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CLINICAL AND EXPERIMENTAL

THE CHOLESTEROL CONTENT OF THE PLASMA IN ARTHRITIS*

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THE extensive literature on blood cholesterol contains few records of studies on cholesterol metabolism in arthritis. Carbohydrate, protein and mineral metabolism have been investigated in detail, the general assumption being that fat (and sterol) metabolism in such cases is normal. Pemberton[1] observed that many patients with arthritis showed a delayed removal of glucose from the blood following its ingestion, and that a low caloric diet had a beneficial influence on the clinical course of the disease; these findings, he believed, indicated that these patients might show some disturbance in fat metabolism. Pemberton and Foster[2] determined the cholesterol and fat content of the whole blood and of plasma in fourteen patients with arthritis and found normal values. The plasma cholesterol in their cases ranged from 68 to 137 mg. per 100 c.c., which in the light of our present knowledge of the normal range for plasma cholesterol, may be considered as definitely low. Gorham and Myers[3] studied the cholesterol content of the blood in five cases of arthritis and found normal values. Bruger and Poindexter[4] reported observations on eleven cases, and they stated that, in general though not consistently, rheumatoid or gonococcal arthritis was associated with hypocholesterolemia and osteoarthritis with hypercholesterolemia.

These latter observations prompted a detailed study of the blood cholesterol in a large number of arthritics. The present investigation includes a study and statistical analysis of the cholesterol partition in the plasma in 92 cases of

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arthritis and a correlation between the sedimentation rate and the cholesterol in a large number of these patients.

MATERIAL AND METHODS

The subjects for this study were obtained from the Arthritis Clinic of the New York Post-Graduate Hospital. Only typical cases of rheumatoid or osteoarthritis were selected, the accepted criteria for their differentiation being carefully observed. In all cases, the duration of the disease varied from three months to ten years.

TABLE I*

VARIATIONS OF THE FREE AND ESTER CHOLESTEROL AND OF THE SEDIMENTATION RATE IN RHEUMATOID AND OSTEOARTHRITIS

CASE NUMBERS	AGE RANGE YR.	SEX		PLASMA CHOLESTEROL RANGE			SEDIMENTATION RATE RANGE MM. PER HOUR
		MALE	FEMALE	TOTAL MG. PER 100 C.C.	ESTERS MG. PER 100 C.C.	ESTERS PER CENT OF TOTAL	
Rheumatoid Arthritis							
1-10	17-55	5	5	100.0-153.8	56.4-107.1	48.5-76.3	13-46
11-20	27-55	3	7	153.8-180.3	74.1-137.9	46.6-76.8	6-47
21-30	21-55	3	7	182.0-226.0	105.4-142.0	51.7-72.0	8-41
31-33	32-55	1	2	230.0-302.5	122.5-178.5	51.0-59.0	14-21
Average	39			175.2	106.8	60.9	25
Osteoarthritis							
1-10	29-60	0	10	150.0-192.3	85.6-127.5	45.9-72.6	5-11
11-20	39-64	3	7	193.3-214.3	97.1-139.9	50.2-69.4	2-22
21-30	39-64	0	10	216.8-235.8	108.4-157.5	49.2-68.8	2-27
31-40	44-69	1	9	238.8-246.8	114.4-158.8	47.6-64.8	2-18
41-50	32-65	1	9	251.5-271.8	118.6-189.4	46.2-75.3	2-25
51-59	42-73	1	8	275.8-416.5	139.9-225.9	42.9-63.7	5-32
Average	51			235.4	140.9	59.4	11

*The data in the above table have been grouped in order to conserve space.

TABLE II

THE TOTAL CHOLESTEROL CONTENT OF THE PLASMA IN NORMAL SUBJECTS AND IN PATIENTS WITH ARTHRITIS: STATISTICAL ANALYSIS

GROUP	NUMBER OF OBSERVATIONS	ARITHMETIC MEAN MG. PER 100 C.C.	STANDARD* DEVIATION	PROBABLE† ERROR OF MEAN	$\frac{d}{\sigma_D}$‡
Normals	33	194.7	29.3	3.42	0.0
Rheumatoid arthritis	33	175.2	39.5	4.60	2.27
Osteoarthritis	59	235.4	45.1	3.69	5.20

*Standard deviation $\sigma = \sqrt{\frac{\Sigma (d^2)}{N}}$

Where $\Sigma (d^2)$ represents the summation of the squares of the individual deviations from the mean, and N the number of determinations.

†Probable error of mean $= 0.6745\frac{\sigma}{\sqrt{N}}$

Where σ represents the standard deviation, and N the number of determinations.

‡$\frac{d}{\sigma_D}$ represents the difference between two means divided by the standard error of the difference.

The standard error of the difference σ_D is calculated from the formula

$$\sigma_D = \sqrt{\frac{\sigma_1^2}{N_1} + \frac{\sigma_2^2}{N_2}}$$

Where σ_1 and σ_2 represent the standard deviations of the two groups and N_1 and N_2 represent the number of determinations in the two groups.

The blood specimens were obtained by venipuncture, usually after breakfast. Free and ester cholesterol were determined by the Bloor-Knudson[5] method. Occasionally, the total plasma cholesterol was determined by the Sackett[6] modification of the Bloor method. Colorimetric determinations were carried out according to the temperature control procedure adopted in this laboratory.[7] The sedimentation rate was determined by the method of Westergren.[8] In many cases, the sedimentation rate and the plasma cholesterol were determined simultaneously; on the other hand, no correlation between cholesterol and sedimenta-

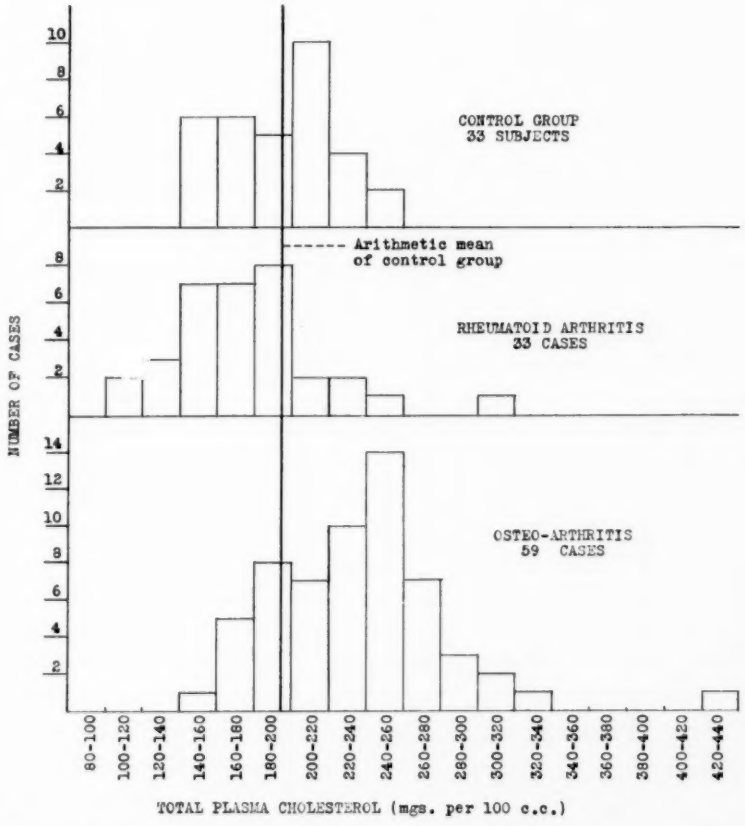

Fig. 1.—Frequency curve. The total cholesterol content of the plasma in normal subjects and in patients with rheumatoid arthritis and osteoarthritis.

tion rate was attempted if more than thirty days intervened between the individual determinations.

In the present investigation, the plasma cholesterol in subjects with arthritis has been compared with cholesterol studies in thirty-three normal subjects, all determinations being carried out under identical conditions in the same laboratory. As has been asserted in previous communications from this laboratory,[4, 9] we believe the cholesterol range in normal subjects to lie between 160 and 230 mg. per 100 c.c. of plasma, values from 230 to 250 mg. are only suggestive of hypercholesterolemia, figures above 250 mg. are considered to be definitely elevated and those below 160 mg. to be distinctly below the normal level.

The formulas used in the statistical analysis of our data are indicated in Table II.

RESULTS

Table I shows the results obtained in 33 cases of rheumatoid arthritis and in 59 cases of osteoarthritis.

In the rheumatoid group the average age was thirty-nine years; 63 per cent of the patients were females. The mean total cholesterol was 175.2 ± 39.5 mg. per 100 c.c. of plasma. When these figures were compared with the control group (see Table II and Fig. 1), the results indicated that 49 per cent of the rheumatoid cases gave normal values, 39 per cent showed hypocholesterolemia,

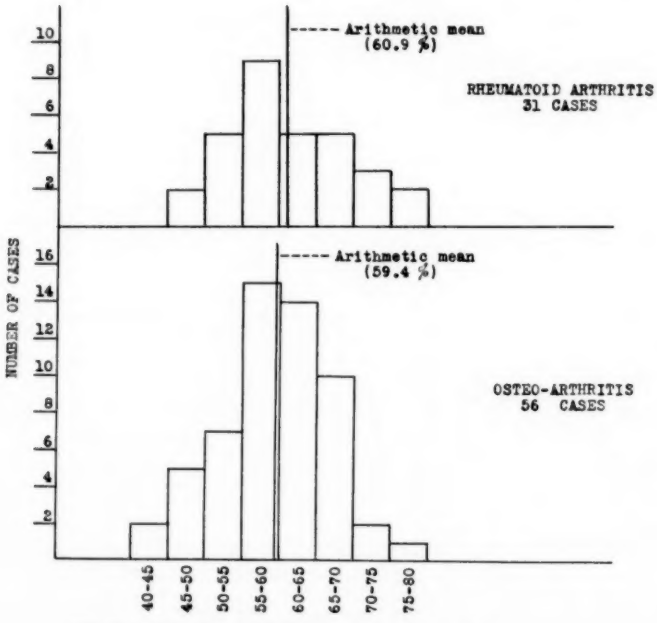

Fig. 2.—Frequency curve. The per cent of esters of the total plasma cholesterol in patients with rheumatoid arthritis and osteoarthritis.

and 12 per cent hypercholesterolemia. The esterified cholesterol showed a normal relation to the total cholesterol varying from 46.6 per cent to 76.8 per cent with a mean of 60.9 per cent. No absolute correlation was observed between the total plasma cholesterol and the sedimentation rate.

In the osteoarthritis group the average age was fifty-one years; 90 per cent of the patients were females. The mean total cholesterol was 235.4 ± 45 mg. per 100 c.c. of plasma. Sixty-two per cent of the cases had hypercholesterolemia, 35 per cent gave normal values, and only 3 per cent showed hypocholesterolemia. The cholesterol esters in this group also showed a normal relation to the total cholesterol, varying from 42.9 per cent to 75.3 per cent with a mean of 59.4 per cent. Here again, no definite correlation was observed between the sedimentation rate and the total blood cholesterol.

Table II presents a statistical analysis obtained in the three groups of subjects. This analysis is included in the present study to show that the results derived from this investigation are at least suggestive. The arithmetic means and the standard deviations indicate the trend, the plasma cholesterol tends to be low in rheumatoid arthritis and elevated in osteoarthritis. That these results, especially those obtained in the osteoarthritis group, are really significant is evident from the final calculations; the difference between the means of the normal group and the rheumatoid group divided by the standard error of the difference of these means gives a value of 2.27. This variation from normal is suggestive but not definite. The same calculations applied to the normal and osteoarthritis group show a figure of 5.20, indicating a significant variation from normal.

Figs. 1 and 2 present frequency curves of the data given above. They are self-explanatory and require no further elucidation.

DISCUSSION

The etiology of rheumatoid arthritis is unknown; the more recent clinical and laboratory data point to its infectious origin. The type of infection and its mode of action are still in dispute; the pathologic findings, however, would suggest either a hematogenous infection or an allergic response as the prime factor in the causation of this type of arthritis. The observation recorded in this paper that patients with rheumatoid arthritis tend to show a low blood cholesterol supports the infectious theory, since other acute infections are accompanied by hypocholesterolemia (Denis,[10] Kipp,[11] Stepp,[12] Rosen and Krasnow,[13] Eichelberger and McCluskey,[14] Henning[15]).

The etiology of osteoarthritis, likewise, is not definitely known. The consensus of opinion is that the pathologic manifestations suggest the degenerative origin of this disease. The tendency to an elevated blood cholesterol in this condition supports this view, since other degenerative diseases are usually accompanied by hypercholesterolemia (Bruger and Poindexter[4]).

The relation of free to ester cholesterol in the plasma of arthritics is not disturbed. The mean percentage of esters of the total plasma cholesterol in the two groups of arthritis (60.9 per cent in rheumatoid arthritis and 59.4 per cent in osteoarthritis) is almost identical with that given by Bloor and Knudson[16] (58 per cent) for normal subjects.

The sedimentation rate in rheumatoid arthritis is usually increased, the rapidity of sedimentation being roughly proportional to the activity of the disease. We hoped to observe an inverse ratio between the plasma cholesterol and the sedimentation rate in these cases, since both determinations appeared, at first hand, to be an index of the severity of the disease. As stated above, no such correlation was observed, although one might venture to say that extremely low plasma cholesterols (as we have encountered in the acute phases of rheumatoid arthritis and in acute rheumatic fever) are usually accompanied by an increased rate of blood cell sedimentation. The converse, that is, a high cholesterol and a normal or low sedimentation rate was not obvious from our figures in the rheumatoid group, though suggestive in the osteoarthritis group.

In the first analysis, two factors may account for the elevated blood cholesterol in osteoarthritis. First, these patients are usually obese, and second, they generally fall into an older age group. In a previous communication from this laboratory,[4] we have shown that uncomplicated obesity (that is, obesity in which evidence of any complicating degenerative disease is lacking) is associated with a normal plasma cholesterol. Again, there are few pertinent studies in the literature which point to an increased blood cholesterol in the aged. Parhon and Parhon[17] are of the opinion that in human subjects there is only a slight increase in the blood cholesterol with advancing years. In very old people (over seventy years of age) they observed a definite hypercholesterolemia. In a group of fourteen normal subjects reported by Gorham and Myers,[3] three were fifty-one, sixty-five, and fifty-five years old, respectively, and the blood cholesterol in these three patients ranged from 130 to 170 mg. per 100 c.c. We have frequently encountered normal plasma cholesterols in old subjects and are of the opinion that the factor of age alone in no way influences the level of the blood cholesterol in human subjects. When arteriosclerosis supervenes, however, the cholesterol content of the plasma may increase, but even the advent of this condition is not invariably accompanied by an elevation of the blood cholesterol (Gorham and Myers,[3] Peters and Van Slyke[18]). It seems correct to conclude, therefore, that the two factors of obesity and of age may be eliminated as possible causes for the increased blood cholesterol observed in patients with osteoarthritis.

SUMMARY AND CONCLUSIONS

The total cholesterol content of the plasma tends to be decreased in rheumatoid arthritis and elevated in osteoarthritis. These findings lend added proof to the theory that rheumatoid arthritis is an infectious disease and osteoarthritis a degenerative one. The relation of free to ester cholesterol in the plasma is not disturbed in patients with arthritis; the cholesterol partition of the plasma in all cases of arthritis approaches closely to that observed in normal subjects. There is no absolute correlation between the sedimentation rate and the total blood cholesterol in patients with arthritis; at times, very low plasma cholesterols are associated with elevated sedimentation rates.

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OBSERVATIONS ON THE INDICAN TEST ON THE BLOOD AND URINE IN RENAL INSUFFICIENCY*

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IN 1911, Obermeyer and Popper[1] first called attention to the fact that indican is increased in the blood of patients with uremia. This was soon confirmed by the observations of Tschertkoff[2] and others, whose investigations showed that a quantitative determination of indican in the blood could be used as a valuable aid in detecting renal insufficiency. Thus Baar,[3] in a monograph on indicanemia, asserts that indican retention is the most accurate of all evidences of disturbed renal function. He based this assertion on the observation that in an individual with normal kidneys the blood indican remains constant regardless of the amount of indican absorbed from the intestines,[4] whereas if the kidneys are impaired, a marked indicanemia occurs, even in the absence of intestinal putrefaction. Rosenberg[5] reports indican values as high as 7 mg. per 100 c.c. of blood in patients with renal insufficiency. (The normal figures are 0.026 to 0.085 mg. per 100 c.c. of blood.) The frequent association of indicanemia with renal insufficiency has also been pointed out by Schilling and Holzer,[6] Becher,[7] Eufinger and Bader,[8] Becher, Litzner, and Doenecke,[9] Muto,[10] Harrison and Bromfield[11] and others.

The observations of Krociewicz[12] and others[13, 14] point to the possibility that the quantitative estimation of the blood indican may be of aid in the diagnosis of renal disease even before retention of nonprotein nitrogen begins. It has also been claimed that, since indican accumulates in the blood only when there is destruction of renal parenchyma, the test may be used as an

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aid in differentiating true renal insufficiency from conditions with nonprotein nitrogen retention which is not due to renal disease primarily. Thus, the observations of Monias and Shapiro[15] have led them to conclude that the accumulation of indican in the blood is a sensitive and specific test of absolute renal insufficiency, reliable in both diagnosis and prognosis. Salvini[16] has recently arrived at a similar conclusion in studying a series of cases showing an increased indicanemia following operation for tumors of the uterus and ovaries.

Should the above observations be confirmed, the clinician would be provided with a much desired test for the diagnosis of early renal disease. The importance of such a test is obvious, since the various tests for renal insufficiency at present employed are of value in detecting only advanced renal disease. It is only occasionally that they serve as an aid in diagnosis of early renal impairment.

Following the report on indicanemia in renal disease by Monias and Shapiro in 1930, we made quantitative determinations on the blood indican in a series of cases of renal insufficiency. It soon became apparent that not all individuals with renal dysfunction and pronounced nonprotein nitrogen retention showed an increase in the blood indican. This observation showed the necessity for further investigation of this subject. Routine quantitative indican determinations were, therefore, performed on all bloods received in our laboratory for chemical examination and, at the same time, quantitative indican determinations were made on the urine of each of these patients.* A record was also made of the results of other tests of renal function which were performed on these patients (urinalyses, phenolsulphonephthalein excretion and the diazo reaction on the blood) and of any significant clinical signs of renal disturbances whenever such signs were present. Three hundred cases were analyzed in all, of which 241 showed no clinical or laboratory evidence of renal dysfunction. Of the remaining 59 cases some showed varying degrees of renal insufficiency, clinically, and all showed an increase of one or more of the nonprotein nitrogen constituents of the blood (urea nitro-

*Jolles'[17] modification of the Obermeyer and Popper method was employed in this study, using the artificial standard of Monias and Shapiro. The technic is as follows: Five cubic centimeters of serum are placed in an Erlenmeyer flask and to this is added an equal quantity of distilled water. An equal quantity (10 c.c.) of trichloracetic acid (20 per cent) is then added and the flask is rotated for about two minutes and then allowed to stand for about five minutes. The contents are then filtered through a funnel into a 100 c.c. graduated cylinder. Ten drops of a 5 per cent alcoholic solution of thymol are added to the clear filtrate to which concentrated fuming, chemically pure, hydrochloric acid containing 0.5 per cent ferric chloride is added in a quantity equal to that of the amount of filtrate obtained. The mixture is well shaken and allowed to stand for two hours. Then 5 c.c. of chemically pure chloroform are added and the mixture is shaken for two or three minutes and allowed to stand. The chloroform which settles at the bottom with the dissolved indigo is now pipetted off and the intensity of its coloration is compared with the artificial standard (6 capillary drops of a 1 per cent aqueous solution of gentian violet, 3 drops of a 1 per cent aqueous solution of eosin, 10 c.c of 94 per cent alcohol and 30 c.c. of distilled water) which corresponds in tint and intensity to the original standard of 5 mg. of indican in 100 c.c. of chloroform. When necessary, dilutions of the standard are made with water and the dilutions taken into account in calculating the results.

The calculations are made from the formula:

$$\frac{\text{Reading of the standard} \times 0.5}{\text{Reading of the unknown}} \times 10 = \text{mg. of indican per 100 c.c. of blood.}$$

The method employed for the quantative determination of the indican in the urine is the same as for blood.

Note: Recently Schlierbach[18] recommended the use of Palfrich's step photometer for quantitative indican determinations which may be controlled by Lange's electric light colorimeter. The advantage claimed for this test is that it obviates the necessity for using photosensitive comparative solutions.

gen of 30 mg. or more and creatinine of 3 mg. or more per 100 c.c. of blood was considered evidence of retention).

The former group (cases with normal renal function and no retention of nonprotein nitrogen) can be dismissed with the statement that in no instance was a definite increase of indican found in the blood.

The latter group (cases with definite retention of nonprotein nitrogen in the blood and with or without clinical evidence of renal disease) was divided according to the blood indican content, into two subgroups, one consisting of 21 cases with normal and the other, of 38 cases, with increased blood indican values. The results of this analysis can be studied best from Tables I and II.

DISCUSSION OF TABLE I

A study of Table I reveals the most significant findings. Four, or about 20 per cent of the 21 cases in which the indican values of the blood were normal (Cases 34, 68, 72, and 94), showed marked renal pathology at autopsy. In Case 34 the amount of renal parenchyma which remained normal was very meager, and in Cases 72 and 94 an arteriosclerotic process had destroyed the major portion of renal parenchyma. In Case 72 there were diffuse vascular lesions in the kidneys and large portions of renal parenchyma had been destroyed by infarcts. Thus in four proved cases of advanced renal pathology the blood indican values were normal. It is significant, however, that in none of these cases was the nonprotein nitrogen retention excessive. The urea nitrogen values ranged only from 31.2 mg. to 52.13 mg. and the creatinine only from 2.4 mg. to 2.9 mg. per 100 c.c. of blood. It is also noteworthy that the Diazo test on the blood was negative in all of these 4 cases as well as in all except one (Case 97) of the remaining 17 cases of this group. This incidentally confirms a previous publication by one of us (P) on the value of the Diazo test in nephritis,[19] where it was pointed out that the test is not always positive even in cases of advanced renal pathology.

In six other cases (Cases 30, 49, 57, 75, 83, and 97) of this group, the diagnosis of renal disease was made from clinical signs, irrespective of the definite nonprotein nitrogen retention which was present in all of them, yet in no instance was the blood indican increased. Up to the time of writing two of these patients had died, one from diabetes and chronic nephritis and the other from cardiac decompensation and uremia. The rest were discharged either as cured or improved.

The remaining eleven cases (Cases 56, 58, 59, 62, 64, 77, 84, 87, 95, 101, and 105) of this group consisted of miscellaneous conditions. Although in none of those instances was there sufficient clinical evidence for a definite diagnosis of renal insufficiency, the persistent albuminuria and the moderate nonprotein nitrogen retention which was found in all of these cases suggested renal dysfunction. (Necropsy was performed in only one of these cases and revealed no evidence of renal pathology.)

From an analysis of the above data it is quite apparent that at least in some instances the absence of an increased indicanemia does not exclude the existence of early or even advanced renal disease. This observation, of course,

TABLE I

CASES WITH NORMAL INDICAN VALUES IN THE BLOOD

CASE	MG. PER 100 C.C.				OTHER TESTS OF RENAL FUNCTION			DIAGNOSIS ON DISCHARGE	POSTMORTEM FINDINGS AND OTHER COMMENTS
	BLOOD			URINE	BLOOD	URINE			
	UREA N.	CREAT.	INDICAN		DIAZO	P. S. P.	ALBUMIN		
30	42.6	3.5	0.08	3.9	Neg.	Traces (2 hr.)	3+	Pyelonephritis	Recovered after drainage. (No record of subsequent chemical analysis)
34	31.2	2.4	0.02	5.2	Neg.		3+	Coronary thrombosis	Autopsy: One kidney almost replaced by fibrosis, with atrophy of its ureter. Other kidney showed marked arteriosclerosis
49	47.0	3.0	Traces		Neg.		3+	Chr. nephritis. Diabetes	Died
56	47.0	1.6	Traces	Traces	Neg.	66%	4+	Coronary closure	Improved
57	59.3	3.0	0.06	4.7	Neg.	8%	3+	Nephrosclerosis	Improved
58	47.0	2.3	0.03	3.6	Neg.		Neg.	Acute appendicitis	Recovered
59	36.3	2.3	0.02	3.9	Neg.		4+	Gen. arteriosclerosis	Unimproved
62	47.0	2.2	0.03	3.2	Neg.		3+	Ca. of bladder. Chr. bronchitis. Gen. art. sclerosis	Died
64	50.0	2.9	Traces	9.5	Neg.		4+	Cirrhosis of liver	Died
68	52.3	2.9	0.05	2.9	Neg.	39%	4+	Periarteritis nodosa. Hypertension	Autopsy: Periarteritis nodosa with infarcts in kidneys
72	35.0		0.08	7.2	Neg.		4+	Nephrosclerosis. Cardiac decompensation	Autopsy: Coronary sclerosis, nephrosclerosis
75	33.0	1.7	Traces	1.0	Neg.		4+	Glomerulonephritis	Improved
77	65.0	4.0	Traces	6.4	Neg.		4+	Hypertrophy of prostate	Improved
83	36.0	2.5	Traces	4.2	Neg.		3+	Chronic nephritis	Improved
84	32.0	1.7	Traces	1.8	Neg.	Traces	4+	Pneumonia	Cured
87	39.4	3.0	0.04	3.4	Neg.		4+	General arteriosclerosis	Died
94	34.0	2.9	Traces	3.4	Neg.		3+	General arteriosclerosis. Nephrosclerosis	Autopsy: Nephrosclerosis; general arteriosclerosis
95	61.0	4.3	0.08	5.2	Neg.		3+	Subacute bacterial endocarditis	Died
97	44.0	2.4	Traces	2.1	Pos.		4+	Cardiac decompensation. Uremia	Died
101	46.1	3.1	Traces	5.2	Neg.		2+	Hypertension arteriosclerosis	Died
105	53.6	3.7	0.02	3.9	Serum jaundiced		Negative	Choledocholithiasis and liver necrosis	Autopsy: Kidneys normal. The figures given here are from tests performed three days antemortem. About a month previously the findings were normal

TABLE II

CASES WITH INCREASED INDICAN VALUES IN THE BLOOD

TOTAL NUMBER OF CASES	MG. PER 100 C.C.				OTHER TESTS OF RENAL FUNCTION			DIAGNOSIS WITH NUMBER OF CASES OF EACH	RESULTS
	BLOOD			URINE	BLOOD	URINE			
	UREA N.	CREAT.	INDICAN		DIAZO	P. S. P.	ALBUMIN		
38	Average 57.1 Range 19.6-250.0	Average 5.9 Range 1.7-25.0	Average 2.1 Range 0.1-7.4	Average 4.3 Range traces—12.5	Positive in all except Case 50	Traces (test was performed in only 12% of these cases)	Average 3+ Range 1+ to 4+	Chronic glomerulonephritis 22 Nephrosclerosis 5 Acute glomerulonephritis 2 Urinary obstruction { Hypertrophied prostate 2 { Vesical calculi 1 Polycystic kidneys 2 Hypertension 3 Subacute bacterial endocarditis 1	Died 24 cases Improved 12 cases Unimproved 2 cases

militates against the reliance which has been placed on this test as a means of diagnosis of incipient or even of advanced renal pathology. Thus, in the four cases cited above (Cases 34, 68, 72, and 94), the moderately increased nonprotein nitrogen values of the blood, together with the other tests of renal function, proved to be more informative than did the blood indican determinations.

DISCUSSION OF TABLE II

It was stated above that early in the course of this investigation it had been observed that an increase in the blood indican was not found in any of the cases with normal nonprotein nitrogen values. Neither was an increased blood indican found in any of the cases in Table I, where, as was just pointed out, an increased indicanemia was expected, since all of these cases showed evidence of renal insufficiency varying in degree from mild to fatal forms. It was, therefore, deemed advisable to tabulate separately all the cases which did show an increase in the blood indican and thereby permit of a more accurate comparative study of the two groups. In Table II, therefore, are recorded only those cases which showed an increase in the blood indican. A study of this table reveals that with one minor exception, all of these cases of this group showed marked nonprotein nitrogen retention in the blood, positive blood diazo reactions, poor phenolsulphonephthalein excretion, marked and persistent albuminuria, and clinical evidence of advanced renal disease. In other words, an increase in blood indican occurred only in cases with definite nonprotein nitrogen retention and other clinicopathologic evidences of renal pathology. Of the 38 patients examined in this group, 22 or about 60 per cent were cases of chronic glomerulonephritis. The rest consisted of the following: 5 cases of nephrosclerosis; 3 cases of so-called essential hypertension terminating in uremia; 2 cases of acute glomerulonephritis, one of which was superimposed on a chronic glomerulonephritis; 3 cases of urinary obstruction, two due to hypertrophy of the prostate and one due to vesicle calculi; 2 cases of polycystic kidneys and one case of subacute bacterial endocarditis. Twenty-four, or nearly 63 per cent, of these patients died after a course of a number of weeks or days at the hospital. Postmortem examinations were performed in two of the cases, one a case of nephrosclerosis and the other a case of chronic glomerulonephritis. In both instances the findings confirmed the clinical impression, marked destruction of renal parenchyma having been found in both. It is of interest to note that in a number of instances, while the retained nonprotein nitrogen of the blood remained constant or was diminishing, the clinical condition of the patient was rapidly progressing to a fatal termination. In these instances it was observed that while the indican of the blood increased the indican of the urine diminished. Findings in Case 32 serve to illustrate this observation (Table III).

In cases in which the urine indican fails to decline synchronously with a rise in the blood indican, a fatal prognosis cannot be made with nearly as much certainty. A persistent increase in the urine indican, on the contrary, signifies renal ability to excrete this toxic substance and, given sufficient time, the excess accumulation of the blood indican may be eventually eliminated.

The group of cases with urinary obstruction deserves special comment. In all of these cases the blood findings, including the indican values, returned

TABLE III

MG. PER 100 C.C.			
BLOOD			URINE
UREA	CREATININE	INDICAN	
125.0	10.0	0.56	9.8
81.3	10.0	5.20	Traces
52.4	10.0	7.20	Traces

to normal soon after the cause of obstruction was removed in each case. Apparently, no permanent renal damage had been produced by the obstruction. The increase of the indican and nitrogenous waste products in the blood was, therefore, only transitory. This observation again illustrates the fallacy of attaching prognostic or diagnostic significance to the results of single blood chemical determinations.

Another observation worthy of comment is that the indican determination may be made on blood secured postmortem and also on blood which had been preserved in a refrigerator for an indefinite period of time. Determinations made on specimens of blood so obtained or preserved yield values which, for practical purposes, are the same as those derived from the examination of blood obtained during life. Control experiments similar to those recently described by one of us (P) in a report on postmortem blood chemistry in renal disease[20] showed that the results of such examinations are reliable and may be used without hesitation when, for various reasons, it may be impossible to obtain fresh blood for analysis.

SUMMARY AND CONCLUSIONS

Quantitative indican determinations were made on the blood and urine of 300 hospital patients, all of whom were observed for evidence of renal disease. Blood urea nitrogen and creatinine determinations and other tests of renal function were made in each case. Based on the results of these examinations the cases were divided into two groups. One group consisted of 241 cases none of which showed any evidence of renal disease. The other group consisted of 59 cases all of which showed definite nonprotein nitrogen retention in the blood and most of which showed also clinical evidence of renal pathology. These 59 cases were subdivided into two subgroups, one consisting of cases with normal, the other with increased blood indican values. The conclusions drawn from this investigation were as follows:

1. Increases in the blood indican values were always accompanied by increases in the blood nonprotein nitrogen content.

2. The blood indican values remained normal in a considerable number of instances in which the nonprotein nitrogen of the blood was definitely increased and in which clinical, laboratory and finally, postmortem examination revealed evidence of severe renal disease.

3. A persistent increase in the blood indican values occurring concomitantly with a persistently diminishing indican content in the urine was found

to be significant of a rapidly fatal prognosis, and in that respect, more reliable than determinations of the nonprotein nitrogen content of the blood. About two-thirds of the patients with a persistent increase in the blood indican died during the course of the investigation.

4. Increases in the blood indican were found to be only temporary in several instances of urinary obstruction. Removal of the cause of obstruction was immediately followed by a return of the blood indican values to normal.

5. Indican determinations may be made on postmortem or preserved blood.

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BACTERIOSTATIC AND BACTERICIDAL STUDIES OF VARIOUS DYES AND ALLIED COMPOUNDS*

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THE indiscriminate use of various compounds in the treatment of tuberculous empyema, and especially the form complicated with pus-forming microorganisms, prompted these studies. Numerous attempts both clinical and experimental have been made to sterilize the pleural cavity, but up to the present not a single known compound has proved of great value in such therapy. It is true there are a number of effective organic mercurial compounds which exert bactericidal properties in vitro, but as soon as they come in contact with body fluids this property becomes nullified, and they are of little value. The demand for an effective drug for such therapy has so increased in recent years, that it could not very well be ignored, but at the outset we fully realized the difficulties intimately connected with it.

A general survey of the subject revealed that a number of compounds with established bacteriostatic and bactericidal properties completely failed to bring about desired results when used for therapeutic purposes. This fairly well signifies that tests as performed today do not give us the true germicidal index of the drug. Usually ordinary beef infusion broth and agar media have been used, and at the best, they are unsuited for a study of this nature. The medium for vitro experiment should be one which will approach the conditions under which the compound will be used. To conform with this we used throughout our studies human serum agar media.

The bacteriostatic property of some 130 dyes and allied compounds were studied, selecting the effective ones for the bactericidal tests. Of the organisms used 10 were gram-positive (staphylococci, pneumococci, and streptococci), and 7 were gram-negative (typhosus, paratyphosus, dysentery, coli and vibrio comma). Several groups contained more than one strain. The object was to find out, for example, if Pneumococcus I, II, and III were equal in their susceptibility to a given drug.

A. ORGANIC DYES

I. *Nitroso Dyes:*

1. Naphthol green B

II. *Nitro Dyes:*

2. Martius yellow
3. Naphthol yellow S

*From the Research and Clinical Laboratory.
Received for publication, August 24, 1934.

III. *Azo Dyes:*

(a) Monoazo.
4. Azo bordeaux
5. Bordeaux red
6. Chrysoidine R
7. Chrysoidine Y
8. Naphthylamine brown
9. Naphthylamine yellow
10. Naphthylamine red

(b) Diazo.
11. Trypan blue
12. Trypan red
13. Vital red
14. Azo blue
15. Chrysamine

IV. *Stilbene Dyes:*

16. Chrysophenine

V. *Triphenylmethane Dyes:*

(a) Acid.
17. Alkali blue
18. Aniline blue
19. Brilliant acid blue
20. Neutral violet

(b) Basic.
21. Brilliant green
22. Gentian violet
23. Methyl violet 6B
24. Iodine green
25. Methyl green
26. Basic fuchsin
27. Para rosaniline base
28. Para rosaniline sulphate
29. Para rosaniline acetate
30. Rosaniline hydrochloride
*31. Para rosaniline salicylate
*32. Isoamyl-para rosaniline iodide
33. Ethyl violet
*34. Ethyl violet bromide
*35. Ethyl violet iodide
*36. n-Propyl violet (homologue of ethyl violet)

VI. *Xanthene Dyes:*

(a) Pyronines.
37. Acridine red

(b) Fluoresceins.
38. Phloxine

(c) Rhodamines.
39. Rhodamine B
40. Rhodamine G
41. Rhodamine 6G

VII. *Acridine Dyes:*

42. Acridine yellow
*43. Acridine yellow salicylate
44. Acridine orange
45. Proflavine
46. Neutral acriflavine
47. Acriviolet

VIII. *Quinoline Dyes:*

48. Quinoline yellow
49. Ethyl red
50. Ethyl cyanine T
51. Orthochrome T bromide

*Prepared in our laboratory.

52. Pinacyanole
53. Pinaflavol
54. Quinaldine red

IX. *Azine Dyes:*

(a) Chinoxalines.
55. Pinacryptol green

(b) Safranines.
56. Methylene heliotrope
57. Methylene violet 3 RA extra
58. Safranine purple M
59. Safranine Y
60. Indoine blue B B
61. Iris violet
62. Induline scarlet

(c) Nigrosines.
63. Nigrosine

X. *Oxazine Dyes:*

64. Nile blue
65. Gallocyanine
66. Alizarine green G
67. Cresyl violet

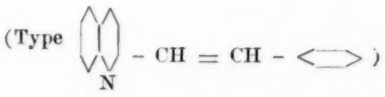
XI. *Thiazine Dyes:*

68. Methylene blue
69. New methylene blue N
70. Thionine
*71. Ethylene blue
*72. n-Propylene blue } homologues of methylene blue
*73. n-Butylene blue

XII. *Anthraquinone Dyes:*

74. Alizarine blue S
75. Alizarine red S

B. STYRYLQUINOLINE COMPOUNDS

(Type )

XIII.

(a) Hydrochlorides.
*76. Benzylidene quinaldine
*77. Benzylidene-4-methyl quinaldine
*78. Benzylidene-6-methyl quinaldine
*79. Cinnamylidene quinaldine
*80. o-Aminocinnamylidene quinaldine
*81. Benzylidene-6-diethylamino quinaldine
*82. Piperonylidene-6-diethylamino quinaldine
*83. o-Hydroxybenzylidene quinaldine
*84. p-Hydroxybenzylidene quinaldine
*85. 2(β-Furfuryl-vinyl) quinoline

(b) Methochlorides.
*86. Benzylidene-6-methyl quinaldine
*87. Cinnamylidene quinaldine
*88. Cinnamylidene-6-methyl quinaldine
*89. o-Nitrocinnamylidene quinaldine
*90. o-Chlorobenzylidene quinaldine
*91. o-Chlorobenzylidene-6-methyl quinaldine
*92. p-Dimethylaminobenzylidene-6-methyl quinaldine methiodide

*Prepared in our laboratory.

C. ANIL QUINOLINE COMPOUNDS

(Type)

XIV.

*93. 2(p-Dimethylamino anil)-β-naphthoquinaldine methochloride
*94. 2(p-Dimethylamino anil)-β-naphthoquinaldine methonitrate
*95. 2(p-Dimethylamino anil)-β-naphthoquinaldine methacetate
*96. 2(p-Dimethylamino anil)-β-naphthoquinaldine methosulphate
*97. 2(p-Diethylamino anil)-β-naphthoquinaldine methacetate
*98. 2(p-Dimethylamino-o-methyl anil)-β-naphthoquinaldine methosulphate
*99. 2(p-Dimethylamino-o-methyl anil)-β-naphthoquinaldine methacetate
*100. 2(p-Dimethylamino-o-chloro anil)-β-naphthoquinaldine methacetate
*101. Condensation product of β-naphthoquinaldine methosulphate and 6-Nitroso-N-ethyl-tetrahydroquinaldine
*102. Condensation product of β-naphthoquinaldine methiodide and 6-Nitroso-N-ethyl-tetrahydroquinaldine
*103. Condensation product of β-naphthoquinaldine methacetate and 6-Nitroso-N-ethyl-tetrahydroquinaldine
*104. Condensation product of β-naphthoquinaldine methacetate and 6-Nitroso tetrahydroquinaldine
*105. 2(p-Monoethylamino-m-methyl anil)-β-naphthoquinaldine methacetate
*106. 2(p-Dimethylamino anil)-4-ethoxy-α-naphthoquinaldine methiodide
*107. 2(p-Dimethylamino anil)-4-ethoxy-α-naphthoquinaldine methosulphate

D. QUINOLINE DERIVATIVES

XV.

108. Quinosol
*109. 8-Hydroxyquinaldine hydrochloride
*110. 2-Ethyl-3-methyl-4-anilino-quinoline hydrochloride
*111. 2-Propyl-3-ethyl-4-anilino-quinoline hydrochloride
*112. 2(Phenylisoaminomethyl)-3-chloro-4-anilino-quinoline hydrochloride
*113. 2(Chloromethyl)-3-ethyl-4-anilino-quinoline hydrochloride
*114. Quinaldyl-tetraethyldiaminodiphenylmethane hydrochloride
*115. Quinaldyl-tetraethyldiaminodiphenyl carbinol hydrochloride
*116. 2-Trichloroethylidene quinaldine hydrochloride
*117. Quinoline-2-acrylic acid
*119. 2-Crotonylidene quinaldine hydrochloride
*120. Methiodide of 2-β-naphthoquinaldehyde phenylhydrozone
*121. 2(Chloromethyl)-3-chloro-4-(p-toluidino)-6-methyl quinoline hydrochloride
*122. 5, 7-Dinitro-8-hydroxyquinoline
*123. 5, 7-Diamino-8-hydroxyquinoline hydrochloride

*Prepared in our laboratory.

E. ALKALOIDS AND DERIVATIVES

XVI.

124. Harmine
125. Isoquinine
126. Aminohydroquinine
127. Ethylapoquinine
*128. 5-Amino-8-phenylazo-dihydroquinine
*129. 5-Amino-8-(2-chlorophenyl)-azo-dihydroquinine
*130. 5-Amino-8-(3-chlorophenyl)-azo-dihydroquinine

Methods Employed.—The bacteriostatic tests were carried on Petri plate media. Churchman's[1] method of divided plates, that is, one-half containing medium with dye and the second half acting as control, proved satisfactory. The original method as introduced by Churchman was slightly modified and proved satisfactory for preparation of the plates. Small strips of stiff cardboard 0.5 cm. wide were cut slightly longer than the inside diameter of the dish. When placed crosswise in the bottom part of the dish, there will be two compartments. If the strip is of the proper length, it will bulge slightly in the center with spring effect to keep itself in place. The cover is put on and the dish sterilized at 150° C. for two hours.

To melted pneumococcus agar of pH 7.6, which has been cooled to 45° C., pleural fluid to 10 per cent was added and kept at this temperature until used.

Into one compartment in the Petri plate prepared as described about 10 c.c. of the melted medium is poured, the cardboard division preventing the medium running through the entire dish. After solidification a straight diametrical cut is made in the medium with a sterile platinum spade, and with a sterile forceps the cardboard is lifted, carrying away some of the adhered medium. This manipulation results in a perfect half plate which is the control.

To a definite amount of melted serum agar, the dye is added to make a dilution ranging from 25,000 to 200,000 for gram-positive organisms, and 5,000 to 100,000 for the gram-negative organisms. First the dyes were dissolved either in water or 95 per cent alcohol and then added to the melted serum agar to the desired dilution, and 10 c.c. of this poured into the second compartment following the same method. The plates will be in perfect halves, one containing the serum agar control and the other the medium with the dye to be studied.

In order to conserve media as much as possible, six or seven streaks were made on each plate. To control the proper distribution of the streak, the bottom of each plate was ruled with a special ruler which consisted of a wire (No. 14 gauge) ring with soldered cross pieces. The ruling was done with India ink. For inoculation the lines can be followed, and there is no difficulty in making six or seven streaks on a single plate. All cultures to be studied were made on pneumococcus serum broth of pH 7.6, and for uniformity, 0.1 c.c. of inoculum was used. The inoculation of the plates was made from an eighteen-hour growth with a platinum loop of 3 mm. in diameter, streaking across the two compartments, and having as a guide the lines on the bottom of the plate.

*Prepared in our laboratory.

The plates were incubated at 37.5° C. and the first reading made at twenty-four hours. The results recorded in the table were the forty-eight-hour observations, which were made in triplicate.

In order to determine the value of a compound, in anticipation of its practical application in therapy such as we were interested in, the following gram-positive and gram-negative organisms were used. Gram-positive: *Staphylococcus pyogenes aureus*, Pneumococcus I, II, III, *Streptococcus hemolyticus, nonhemolyticus, scarlatina, viridans, erysipelatis* and *faecalis*. Gram-negative: *Bacillus typhosus, paratyphosus A and B, dysentery Shiga, Flexner and Sonne, coli*, and *vibrio comma*.

The results are summarized in Tables I, II, and III. To save space in the table, we have omitted 000 from the figures, therefore each number represents thousands.

Out of 130 compounds studied, 51 were effective for gram-positive, and 23 for gram-negative organisms. Studying the tables the reader will notice that our results are considerably lower than those reported by other investigators. This discrepancy in all probability is due to the difference of media. At best, on ordinary culture medium, many organisms propagate only spar-

TABLE I*

BACTERIOSTATIC STUDIES—GRAM-POSITIVE ORGANISMS

LAB. NO.	COMPOUND	STAPH. P. AUREUS	PNEUMO. I	PNEUMO. II	PNEUMO. III	STREP. HEMOLYTICUS	STREP. NONHEMOLYTIC.	STREP. SCARLATINA	STREP. ERYSIPELATIS	STREP. VIRIDANS	STREP. FAECALIS
21	Gentian violet	50	50	50	50	50	25	25	25	25	--
22	Ethyl violet	50	50	100	50	50	50	50	50	50	25
25	Brilliant green	100	200	200	200	100	50	50	50	50	25
26	Basic fuchsin	25	50	100	50	50	50	50	50	50	25
27	para Rosaniline acetate	50	100	100	100	50	50	50	50	50	--
28	para Rosaniline base	25	50	50	50	50	25	25	25	25	--
29	para Rosaniline sulphate	--	25	50	50	50	50	50	50	50	--
30	Rosaniline hydrochloride	--	50	50	50	50	50	50	50	25	--
31	para Rosaniline salicylate	--	50	50	50	25	25	25	25	25	--
32	Ethyl violet bromide	--	25	25	25	25	25	25	25	25	25
33	Ethyl violet iodide	--	25	50	25	25	25	25	25	25	--
34	Isoamyl-para rosaniline iodide	--	25	25	25	25	25	25	25	25	--
35	Methyl violet 6B	100	150	200	150	50	50	50	50	50	25
36	n-Propyl violet	100	150	150	150	50	50	50	50	50	25
41	Rhodamine 6G	25	25	25	25	25	25	25	25	25	--
42	Acridine yellow	25	100	100	100	100	25	25	25	25	--
44	Proflavine	50	100	100	50	100	50	50	50	50	25
45	Neutral acriflavine	100	100	100	100	100	100	100	100	100	25
46	Acriviolet	50	100	100	100	100	50	50	50	50	25
54	Orthochrome T bromide	25	25	25	25	25	25	25	25	25	--
55	Pinacryptol green	--	50	50	50	50	25	25	25	--	--
56	Methylene heliotrope	50	50	50	50	50	50	50	50	50	25
57	Methylene violet 3 RA extra	100	150	150	50	25	25	25	25	25	--
59	Safranine Y	25	50	50	50	25	25	25	25	25	--
60	Indoine blue B B	100	200	200	150	50	25	25	25	25	--
61	Iris violet	--	50	50	50	25	--	--	--	--	--
64	Cresyl violet	100	150	150	150	50	50	50	50	150	25
65	Nile blue	--	25	25	25	--	--	--	--	--	--

*The figures represent thousands.

ingly provided the medium is not enriched with some body fluids. We had no difficulty in observing a profuse growth in the control compartment of the plates with streptococcus and pneumococcus. We may offer at present the following three explanations which, singly or collectively, may have contributed to the low values of the inhibitory properties reported here. (1) The enriched medium allowing the organism uninterrupted propagation, (2) the serum having caused some direct modification of the dye molecule thus mak-

TABLE II*

BACTERIOSTATIC STUDIES—GRAM-POSITIVE ORGANISMS

LAB. NO.	COMPOUND	STAPH. P. AUREUS	PNEUMO. I	PNEUMO. II	PNEUMO. III	STREP. HEMOLYTICUS	STREP. NONHEMOLYTIC.	STREP. SCARLATINA	STREP. ERYSIPELATIS	STREP. VIRIDANS	STREP. FAECALIS
68	Methylene blue	100	200	200	200	100	100	100	100	100	50
69	New methylene blue N	50	100	100	100	50	50	50	50	50	25
71	Ethylene blue	100	300	300	300	150	50	100	100	25	25
72	n-Propylene blue	100	300	300	300	150	50	100	100	25	25
73	n-Butylene blue	100	200	200	200	150	50	100	100	25	25
91	p-Dimethylaminobenzylidene-6-methyl quinaldine methiodide	25	100	100	100	25	--	25	25	25	--
92	o-Chlorobenzylidene-6-methyl quinaldine		25	25	25	25	25	25	25	25	--
93	2(p-Dimethylamino anil)-β-naphthoquinaldine methochloride		100	100	50	50	50	50	50	25	25
94	2(p-Dimethylamino anil)-β-naphthoquinaldine methonitrate		150	200	200	200	50	150	150	25	25
95	2(p-Dimethylamino anil)-β-naphthoquinaldine methacetate		150	200	150	150	150	150	150	50	25
96	2(p-Dimethylamino anil)-β-naphthoquinaldine methosulphate		150	300	150	150	100	100	100	100	50
97	2(p-Diethylamino anil)-β-naphthoquinaldine methacetate		50	150	100	100	25	100	100	25	--
98	2(p-Dimethylamino-o-methyl anil)-β-naphthoquinaldine methosulphate		25	100	100	25	--	25	25	25	--
99	2(p-Dimethylamino-o-chloro anil)-β-naphthoquinaldine methacetate		100	100	50	25	--	25	25	--	--
100	2(p-Dimethylamino-o-methyl anil)-β-naphthoquinaldine methacetate		300	300	300	25	25	25	25	25	--
101	Condensation product of β-naphthoquinaldine methosulphate and 6-Nitroso-N-ethyl-tetrahydroquinaldine		150	150	150	100	25	50	50	50	25
103	Condensation product of β-naphthoquinaldine methacetate and 6-Nitroso-N-ethyl-tetrahydroquinaldine		100	200	150	100	25	50	50	50	25
104	Condensation product of β-naphthoquinaldine methacetate and 6-nitroso tetrahydroquinaldine		50	50	50	50	--	50	50	50	--
105	2(p-Monoethylamino-m-methyl anil)-β-naphthoquinaldine methacetate		50	50	50	50	25	50	50	25	--
106	2(p-Dimethylamino anil)-4-ethoxy-α-naphthoquinaldine methiodide		25	25	25	25	--	25	25	25	--
124	Harmine	--	100	100	100	--	--	--	--	--	--
128	5 Amino-8-phenylazo-dihydroquinine	--	25	25	25	25	--	25	25	25	--
129	5 Amino-8-(2-chlorophenyl)-azo-dihydroquinine	--	150	200	200	150	50	150	100	50	25

*The figures represent thousands.

TABLE III*

BACTERIOSTATIC STUDIES—GRAM-NEGATIVE ORGANISMS

LAB. NO.	COMPOUND	B. TYPHOSUS	B. PARATYPH. A	B. PARATYPH. B	B. DYSENTERY SHIGA	B. DYSENTERY FLEXNER	B. DYSENTERY SONNE	B. COLI	VIBRIO COMMA
23	Iodine green	5	5	5	25	10	10	--	25
24	Methyl green	5	5	5	10	5	5	--	5
25	Brilliant green	50	50	50	100	50	100	50	100
26	Basic fuchsin	10	10	10	25	25	10	10	10
42	Acridine yellow	10	10	10	25	25	25	5	10
44	Proflavine	--	--	--	10	10	10	--	5
45	Neutral acriflavine	--	--	--	50	10	25	--	--
46	Acriviolet	--	--	5	50	50	50	--	10
64	Cresyl violet	--	--	--	10	5	1	--	--
71	Ethylene blue	--	--	--	5	5	--	--	--
72	n-Propylene blue	--	--	--	10	10	--	--	5
73	n-Butylene blue	--	--	--	5	5	--	--	--
95	2(p-Dimethylamino anil)-β-naphthoquinaldine methacetate	200	--	--	150	200	200	--	200
96	2(p-Dimethylamino anil)-β-naphthoquinaldine methosulphate	100	--	--	150	100	150	--	100
97	2(p-Diethylamino anil)-β-naphthoquinaldine methacetate	50	--	--	50	50	50	--	50
98	2(p-Dimethylamino-o-methyl anil)-β-naphthoquinaldine methosulphate	10	--	--	100	--	10	--	5
100	2(p-Dimethylamino-o-methyl anil)-β-naphthoquinaldine methacetate	10	--	--	25	50	25	--	25
101	Condensation product of β-naphthoquinaldine methosulphate and 6-Nitroso-n-ethyl-tetrahydroquinaldine	5	--	--	50	50	50	--	25
103	Condensation product of β-naphthoquinaldine methacetate and 6-Nitroso-n-ethyl-tetrahydroquinaldine	50	--	--	50	50	50	--	50
104	Condensation product of β-naphthoquinaldine methacetate and 6-Nitroso tetrahydroquinaldine	10	--	--	10	25	25	--	25
105	2(p-Monoethylamino-m-methyl anil)-β-naphthoquinaldine methacetate	10	--	--	50	25	25	--	5
107	2(p-Dimethylamino anil)-4-ethoxy-α-naphthoquinaldine methosulphate	25	--	--	25	25	10	--	50
108	Quinosol	50	50	50	100	100	100	25	50

*The figures represent thousands.

ing it ineffective, and (3) the serum acting as protector altering the surface of the bacteria, making them less permeable to the dye.

BACTERICIDAL TEST

A departure from conventional bactericidal methods was adopted in this study. The same gram-positive and gram-negative organisms used in the preceding experiment were tested. As in that experiment 10 per cent serum pneumococcus broth medium of pH 7.6 was used. Culture tubes containing 5 c.c. of this broth were inoculated with 0.1 c.c. of eighteen-hour broth culture and incubated at 37.5° C. for two hours. As most organisms have a lag period of from one to three hours, it seemed advisable to add the compound which was to be investigated after this lag period, thus giving the advantage to the organism and not to the dye. After two hours of incubation of series of tubes,

various amounts of the dye were added, making a dilution of 25,000 to 200,000 for the gram-positive, and for the gram-negative organisms dilutions were from 5,000 to 100,000. The tubes were well shaken and returned for further incubation and readings made at the twenty-fourth hour. At the forty-eighth hour, subinoculations were made from each tube on serum agar plates in order to rule out the possibility of bacteriostasis, which occurs in such experiments after a tube appears to be perfectly clear, when if subcultured colonies develop. To conserve culture media a number of streaks were made on each plate using the method described elsewhere.

The end-results were recorded after the plates had been incubated for forty-eight hours.

The final results were disappointing. The values of the bactericidal activities, of all the compounds used in the bacteriostatic test and summarized in Tables I, II, and III, were approximately one-half and occasionally one-third of those obtained in the bacteriostatic test. The summaries of the tables are omitted as the findings are of no importance.

These studies reveal that the streptococci as a group are much more resistant to bacteriostasis than other gram-positive organisms. *Streptococcus faecalis* is the most resistant, *viridans* is next in order, followed by *nonhemolyticus, scarlatina* and *erysipelatis*. *Streptococcus hemolyticus*, on the other hand, is much more sensitive to the dyes. Next to the streptococci is *Staphylococcus P. aureus*. Pneumococci are more sensitive to bacteriostasis than any of the gram-positive organisms studied. Type II is the most sensitive, type III follows closely and type I reacts less than the others.

Of the gram-negative organisms, the three dysentery organisms studied possessed about similar sensitivities to bacteriostasis. *Bibrio comma* and *B. typhosus* follow very closely. The *B. paratyphosus* A and B and *B. coli* are extremely resistant to the action of the dyes.

During the course of these studies casual observations were made on the comparison of the appearance of the growth in the dye compartment with the growth of the control. Repeatedly we noticed that some dyes exerted only a slight bacteriostatic action—the growth was scanty, here and there sprinkled with single colonies which varied in topography and other physical properties. This was especially pronounced in media containing the dyes of the azine series. This is in accord with many observations reported in the past dealing with forced dissociation of bacteria, which have been fully discussed by Hadley.[2] It seems that, in the future, this phenomenon must be taken into consideration when selecting compounds for studies such as this. We see no reason why the growth should be our only criterion in the evaluation of a drug, and not the variants which are present in the growth.

CONCLUSIONS

In this first contribution, we have presented bacteriostatic and bactericidal studies of 130 dyes and allied compounds, most of which have, in our hands, proved to be of little or no value. The most effective for the gram-positive

organisms were: Groups V, triphenyl methane dyes (basic); VII, acridine dyes; IX, azine dyes, (a) chinoxalines, (b) safranines; X, oxazine dyes; XI, thiazine dyes; XIV, anil quinoline compounds; and a few of XVI, alkaloids and derivatives.

The gram-negative organisms are affected differently. Group V, triphenyl methane dyes (basic), iodine green, methyl green, brilliant green, and basic fuchsin have moderate bacteriostatic and bactericidal properties, and VII, acridine dyes, only feeble activities. Anil quinoline compounds, Group XIV, are the most effective.

At present the most promising is Group XIV, anil quinoline compounds. Many new modifications with and without mercury have been prepared in this laboratory, and the results will be reported later.

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THE BLOOD WASSERMANN TEST IN 500 CASES OF NEUROSYPHILIS WITH POSITIVE CEREBROSPINAL FLUIDS*

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ALTHOUGH many syphilologists and neurologists have expressed the opinion that a negative blood Wassermann frequently is found in neurosyphilis, there have been relatively few clinical studies to support this statement. We here present the results of an investigation of 500 cases of neurosyphilis, chosen on the basis of a positive cerebrospinal fluid in addition to clinical studies which established the diagnosis, as well as the active state of the neurosyphilitic process. In reviewing unselected cases from our record files over a period covering the past eight years, approximately 20 patients with negative cerebrospinal fluids were excluded despite sufficient clinical evidence of syphilis of the central nervous system. This study shows the frequency of a negative, doubtful, and strongly positive blood Wassermann test in the five more common clinical forms of neurosyphilis, all of which showed a positive Wassermann test in the spinal fluid: asymptomatic (297 cases), tabes dorsalis (133 cases), general paralysis (38 cases), taboparesis (19 cases), and meningovascular (13 cases).

The statistical data which are analyzed later were based upon the initial blood and spinal fluid tests taken early during the patient's study in the clinic.

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Only those cases in which the two tests were taken approximately at the same time were included in this series. Notation was made of the amount of treatment the patient had had previous to the time of the lumbar puncture. In subsequent tables the treated and untreated cases are separately considered under their proper headings.

For the past three years (which time interval includes most of the present series of 500 cases), we have employed the standard Kolmer technic for the examination of both blood and spinal fluid. Prior to that time a less sensitive technic was used, but since both the blood and spinal fluid were examined by the same method, the validity of comparative results is not impaired.

The routine study of the spinal fluid has also included a cell count, tests for globulin, and colloidal gold curve determination. Since these latter results, in general, corresponded to the degree of positivity of the Wassermann test in the spinal fluid, they have not been analyzed in this paper.

ASYMPTOMATIC NEUROSYPHILIS

Our study includes 297 cases so classified, all with four-plus cerebrospinal fluid Wassermann tests (Table I).

TABLE I

BLOOD SERUM WASSERMANN	MALES	FEMALES	TOTAL	PER CENT OF ALL 297 CASES	PATIENTS WITHOUT TREATMENT (BOTH MALE AND FEMALE)	PER CENT OF 83 UNTREATED PATIENTS
Negative	85	16	101	33.6	16	19.2
One-plus	5	4	9	3.0	0	0.0
Two-plus	29	10	39	14.0	10	12.2
Three-plus	22	4	26	8.4	7	8.4
Four-plus	74	48	122	41.0	50	60.2
Totals	215	82	297	100.0	83	100.0

Thus, we find that one-third of this large series of cases of asymptomatic neurosyphilis, on their initial examination in our clinic showed a negative blood Wassermann test in the presence of a positive cerebrospinal fluid Wassermann test. An additional nine cases (3.0 per cent) showed only a one-plus blood Wassermann test. Even if one granted that previous treatment might be responsible for the blood Wassermann reversal in a large percentage of cases, it is noteworthy that 19.2 per cent of the 83 patients who gave no history of treatment also showed a negative blood Wassermann test on the initial examination.

The opinions and findings of other workers as to the frequency of a negative blood Wassermann test in asymptomatic neurosyphilis, is shown in Table II.

TABES DORSALIS

The findings in 133 cases of tabes dorsalis, in which every case showed a four-plus Wassermann test in the cerebrospinal fluid are shown in Table III.

It is worthy of comment that 27.8 per cent of all these patients had a negative blood Wassermann on admission, and an additional 7.5 per cent showed an equivocal reaction. Even 21.3 per cent of the untreated patients showed a negative or doubtful blood finding, though all showed clinical evidence of tabes dorsalis as well as strongly positive spinal fluid Wassermann tests.

That tabes dorsalis is frequently associated with a negative blood Wassermann is shown in the uniformity of the reports and opinions of previous investigators (Table IV).

TABLE II

AUTHOR	YEAR	FINDINGS
Fildes, Parnell, Maitland[1]	1919	624 patients, 2 with negative blood in 42 patients with positive spinal fluid
Fordyce and Rosen[2]	1921	14 per cent of late asymptomatic neurosyphilis with negative blood and positive fluid
Worster-Drought, et al.[3]	1922	14 patients, 12 with negative blood
Fuchs[4]	1922	131 patients, 29 per cent with negative blood
Stokes and Brown[5]	1922	112 patients, 72 (69.2 per cent) with positive fluid and negative blood
Stokes[6]	1926	30 to 50 per cent negative blood in latent early neurosyphilis
Moore[7]	1933	"Blood is often reversed and maintained as negative"

TABLE III

BLOOD SERUM WASSERMANN	MALES	FEMALES	TOTAL	PER CENT OF SERIES OF 133 TABETICS	PATIENTS WITHOUT TREATMENT (BOTH SEXES)	PER CENT OF 47 UNTREATED TABETICS
Negative	36	1	37	27.8	9	19.2
One-plus	10	0	10	7.5	1	2.1
Two-plus	13	2	15	11.3	6	12.8
Three-plus	11	0	11	8.2	7	15.1
Four-plus	53	7	60	45.2	24	50.8
Totals	123	10	133	100.0	47	100.0

TABLE IV

AUTHOR	YEAR	FINDINGS
Schaffer[8]	1911	150 cases, negative blood in 31.3 per cent
Nonne[9]	1916	30 to 40 per cent with negative blood
Fordyce[10]	1919	21 cases, 19 per cent with negative blood; 100 per cent had positive spinal fluid
Boyd[11]	1920	30 to 50 per cent with negative blood. Spinal fluid also negative in the same per cent
Gennerich[12]	1921	30 to 40 per cent negative blood
Worster-Drought, et al.[3]	1922	36 cases, five with negative blood
Thaysen[13]	1923	111 cases, 46 with negative blood (42 per cent)
Stokes and Shaffer[14]	1925	30 to 60 per cent with negative blood
Kilduffe[15]	1926	30 per cent negative blood; spinal fluid 100 per cent positive
Fritzsche[16]	1928	15.4 per cent of cases with negative blood and positive fluid
Plaut[17]	1929	20 to 30 per cent negative blood
Geert-Jørgensen, et al.[18]	1932	216 cases, 50 (23.1 per cent) with negative blood and positive fluid
Schamberg and Wright[19]	1932	35 per cent negative blood serum

GENERAL PARALYSIS

The blood Wassermann findings in 38 cases of clinical and seropositive spinal fluid cases of general paralysis are shown in Table V.

TABLE V

BLOOD SERUM WASSERMANN	MALES	FEMALES	TOTAL	PER CENT	CASES WITHOUT TREATMENT (BOTH SEXES)
Negative	6	2	8	21.1	1
One-plus	1	0	1	2.6	0
Two-plus	5	0	5	13.1	0
Three-plus	4	0	4	10.5	0
Four-plus	12	8	20	52.7	6
Totals	28	10	38	100.0	7

In this series we found that 21.1 per cent of cases showing both the clinical picture of general paralysis, as well as positive spinal fluid findings, had a negative blood Wassermann test. Admittedly the number of cases analyzed is too small to warrant any definite conclusion and the series could not be fairly compared with similar studies made on patients suffering from general paralysis whose mental symptoms were severe enough to demand hospitaliza-

TABLE VI

AUTHOR	YEAR	FINDINGS
Marie and Levaditi[20]	1907	39 cases, 41 per cent negative blood and 27 per cent negative spinal fluid
Edel[21]	1908	62 cases, 100 per cent positive blood
Lesser[22]	1908	62 cases, 100 per cent positive blood
Raviart, Breton, Petit[23]	1908	72 cases, 7 per cent negative blood
Stertz[24]	1908	"95 per cent positive fluid and serum even higher percentage positive"
Marinescu[25]	1909	35 cases, 6 per cent negative blood
Nonne and Holzmann[26]	1910	100 per cent positive blood
Darling and Newcomb[27]	1916	69 cases, fluid 100 per cent positive, blood negative in 8 (11.6 per cent) cases
Fordyce[10]	1919	40 cases, 5 per cent negative blood, 100 per cent positive fluids
Plaut[28]	1920	1,400 cases, 8 negative blood, and 14 with weakly positive blood
Kafka[29]	1920	100 cases, 24 with negative blood
Craig[30]	1921	100 per cent positive blood
Nathan and Weichbrodt[31]	1921	150 cases, 27 with negative blood at some stage
Boas[32]	1922	243 cases, 100 per cent positive blood, 94 per cent positive fluid
Eicke[33]	1922	110 cases, 8 with negative blood
Eskuchen[34]	1923	72 cases, 5.5 per cent with negative blood and positive fluid
Menninger and Menninger[35]	1925	166 cases, 7.6 per cent with negative blood and positive fluid
Walther and Abelin[36]	1926	32 cases, 3 with negative blood
Kilduffe[15]	1926	100 per cent positive blood
Fritzsche[16]	1928	4.3 per cent with positive fluid and negative blood
von Rohden, et al.[37]	1931	437 cases, 10 untreated had negative blood
Candler and Mann[38]	1932	173 cases, 11 with positive fluid and negative blood
Peterson[39]	1932	179 cases, 18.5 per cent with negative blood
Salomon[40]	1932	120 cases, 13 with negative blood
Solomon[41]	1932	5 to 10 per cent with negative blood

tion. However, the series is sufficiently large to represent a cross-section of the average experience in private or out-patient clinic practice.

Even though general paralysis has been a special field of investigation as to the relationship of blood and spinal fluid findings, there still remains a wide discrepancy of opinion, as shown in Table VI.

Since the improvement in the technic of the Wassermann test, it is not possible to compare the earlier reports with later studies, and the results since 1920 are probably much more accurate than those published in previous years.

The spinal fluid Wassermann reaction is said by many investigators to be positive in 100 per cent of cases of general paralysis (Eichelberg,[42] Darling and Newcomb,[27] Sabatini,[43] Boyd,[11] Haguenau,[44] Sicard,[45] Cestan and Riser,[46] Nonne,[9] Fordyce,[10] Craig[30]). As is indicated in Table VI, however, there have been many cases with a completely negative spinal fluid reported by reliable workers over a period of years. Furthermore, there have been many unquestioned cases of other types of neurosyphilis reported showing a negative spinal fluid. A classification of such cases was made by Nonne[47] and subsequently expanded by Solomon and Klauder.[48] It has been conclusively shown by Chesney and Kemp,[49] that the *Spirocheta pallida* can be cultivated from the spinal fluid in 14.7 to 26.6 per cent of cases of early syphilis without changes of any kind in the spinal fluid.

TABOPARESIS

Table VII presents the findings of the blood Wassermann test in 19 cases of seropositive spinal fluid cases of taboparesis.

TABLE VII

BLOOD SERUM WASSERMANN	MALES	FEMALES	TOTAL	PER CENT	PATIENTS WITHOUT TREATMENT (BOTH SEXES)
Negative	5	0	5	26.3	1
One-plus	1	0	1	5.2	0
Two-plus	1	0	1	5.2	1
Three-plus	1	0	1	5.2	0
Four-plus	10	1	11	58.1	2
Totals	18	1	19	100.0	4

This survey shows that 26.3 per cent of the 19 patients had a negative blood Wassermann test on their admission to the clinic. Again the number of cases is too small to permit any very definite statement as to the frequency with which a negative blood Wassermann is encountered in this condition. The figures do demonstrate, however, the fact that in dealing with a non-hospitalized group of patients with this type of neurosyphilis, a comparatively large percentage will present a negative blood Wassermann test.

There has been little specific mention of the laboratory findings in taboparesis in the literature. Worster-Drought et al[3] (1922) reported nine cases of taboparesis, four of which had negative blood Wassermann tests. Kilduffe[15] (1926) stated that the blood and spinal fluid are 100 per cent positive in taboparesis.

MENINGOVASCULAR NEUROSYPHILIS

The findings in 13 cases of meningovascular neurosyphilis, all with positive evidence of cerebral vascular damage and with a positive spinal fluid, are shown in Table VIII.

TABLE VIII

BLOOD SERUM WASSERMANN	MALES	FEMALES	TOTAL	PER CENT	PATIENTS WITHOUT TREATMENT
Negative	2	2	4	30.8	0
One-plus	0	0	0	0.0	0
Two-plus	1	1	2	15.4	0
Three-plus	0	0	0	0.0	0
Four-plus	5	2	7	53.8	1
Totals	8	5	13	100.0	1

The series presented is obviously too small to allow any doctrinal conclusions, but the findings remain consistent with those of the other clinical types in showing a surprisingly large percentage of cases with negative blood tests.

We have found no definite reference to the laboratory findings in meningovascular syphilis in the literature. This is probably explained on the basis of varying diagnostic categories, and such cases may have elsewhere been included in the cerebrospinal, the meningitic, or the vascular groups.

FIVE HUNDRED CASES OF NEUROSYPHILIS

The findings for the entire series are shown in Table IX.

TABLE IX

BLOOD SERUM WASSERMANN	MALES	FEMALES	TOTAL	PER CENT OF ALL 500 CASES	PATIENTS WITHOUT TREATMENT (MALE AND FEMALE)	PER CENT OF 142 UNTREATED CASES
Negative	134	21	155	31.0	27	19.0
One-plus	17	4	21	4.2	1	0.7
Two-plus	49	13	62	12.4	17	12.0
Three-plus	38	4	42	8.4	14	9.9
Four-plus	154	66	220	44.0	83	58.4
Totals	392	108	500	100.0	142	100.0

The advantage in grouping all the various clinical types of neurosyphilis in the above table is briefly to demonstrate the remarkable fact that 31 per cent of all such cases presented a negative Wassermann test in the blood, although showing a positive test in the spinal fluid, commonly accepted as evidence of active neurosyphilis. Of the 142 patients without a history of any previous antisyphilitic treatment, a surprisingly high percentage (19.0) showed a negative blood Wassermann test. This might logically lead us to assume that the amount of treatment received might account for about 12 per cent of the negative blood Wassermann tests in the group as a whole. This is probably fallacious, however, since the history in some cases was so vague as to amount and kind of therapy that little dependance could be placed upon

it. Furthermore, a considerable number of patients included in the treated group had previously received such desultory and ineffective treatment as a few mercury inunctions or two or three intravenous injections.

There have been few studies of large groups of neurosyphilis patients reported in the literature with special reference to the blood and spinal fluid laboratory findings. Numerous reports divide their cases into the various diagnostic categories and have been listed in the previous tables. Table X gives studies of neurosyphilis as a group.

TABLE X

AUTHOR	YEAR	FINDINGS
Eskuchen[34]	1923	346 cases, 97 (28.0 per cent) with negative blood and positive spinal fluid
Dattner[50]	1932	900 cases, 20 per cent with negative blood
Haag and Kolbe[51]	1932	321 cases, 18 per cent with positive fluid and negative blood
Geert-Jørgensen, et al.[18]	1933	654 cases, 94 (14.3 per cent) with negative blood and positive fluid

THE KAHN TEST

The Kahn test was routinely made on the blood in each of our cases. It is generally recognized that the Kahn test is more sensitive than the Wassermann test, and hence it is of interest to compare the findings of the Kahn test in the cases with a negative blood Wassermann test in this series. In the 155 cases with a negative blood Wassermann, the blood Kahn was positive in 38 cases. In these 38 instances the Kahn test was "two-plus" in six cases, "three-plus" in 7 cases, and "four-plus" in 25 cases.

Of the entire 500 cases studied, 117, or 23.4 per cent, showed completely negative blood tests, both Wassermann and Kahn.

THE CEREBROSPINAL FLUID WASSERMANN TEST

As previously stated, the present study includes only patients with a positive spinal fluid Wassermann test. An analysis of the titer of fluid positivity discloses the following: of the total of 500 cases 423 showed a four-plus spinal fluid Wassermann reaction with as little as 0.2 c.c. of fluid; of the remaining 77 patients 27 showed a four-plus reaction in the spinal fluid Wassermann with 0.4 c.c. fluid; in 39 cases 0.6 c.c. was the least amount of fluid to show a completely positive Wassermann test; in 11 cases 1 c.c. of spinal fluid was required for a four-plus Wassermann test. It is interesting to correlate these findings with the Wassermann test performed on the blood serum, as shown in Table XI.

TABLE XI

BLOOD SERUM WASSERMANN	SPINAL FLUID FOUR-PLUS IN:		
	0.4 c.c.	0.6 c.c.	1.0 c.c.
Negative	11 cases	20 cases	6 cases
One-plus	2 cases	1 case	0 case
Two-plus	7 cases	1 case	1 case
Three-plus	0 case	2 cases	0 case
Four-plus	7 cases	9 cases	4 cases
Totals	27 cases	39 cases	11 cases

SUMMARY

The problem of the status of the blood Wassermann in 500 cases of clinical neurosyphilis with positive spinal fluids is presented, together with a brief review of the literature.

1. In the entire group of all clinical types of neurosyphilis, 155 (31 per cent) showed negative blood Wassermann tests early in their study at the clinic. Twenty-one patients (4.2 per cent) showed a one-plus blood Wassermann test; 62 patients (12.4 per cent) showed two-plus; 42 patients (8.4 per cent) showed three-plus; and 220 patients (44 per cent) showed a four-plus blood Wassermann test.

2. In 297 cases of asymptomatic neurosyphilis, 101 patients (33.6 per cent) showed a negative blood Wassermann test.

3. In 133 cases of tabes dorsalis, 37 patients (27.8 per cent) showed a negative blood Wassermann test.

4. In 38 cases of general paralysis, 8 patients (21.1 per cent) showed a negative blood Wassermann test.

5. In 19 cases of taboparesis, 5 patients (26.3 per cent) showed a negative blood Wassermann test.

6. In 13 cases of meningovascular neurosyphilis, 4 patients (30.8 per cent) showed a negative blood Wassermann test.

7. The Kahn test was positive in 38 cases showing a negative blood Wassermann.

If this number of cases is deducted from the 155 showing a negative blood Wassermann, there remain 117 patients (23.4 per cent) with both the Kahn and Wassermann tests in the blood negative.

CONCLUSIONS

1. In approximately 30 per cent of cases of active neurosyphilis with positive spinal fluid tests, the blood serum Wassermann test fails to give any indication of this process. In 23 per cent neither the blood Kahn nor Wassermann test is positive.

2. In view of this fact, no case of syphilis, after the early stage, can be regarded as completely studied, accurately diagnosed, or correctly treated, without knowledge of the cerebrospinal fluid findings.

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DIURNAL VARIATIONS IN CONCENTRATION OF RED BLOOD CELLS AND HEMOGLOBIN*

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NUMEROUS data have accumulated over many years on the subject of red cell and hemoglobin values in the peripheral blood stream. Despite earnest and painstaking efforts on the part of many competent workers, the question as to whether significant physiologic diurnal variations occur is still in the realm of controversy. In 1923, Rabinowitch[1] reported hemoglobin variations of from 1.1 to 26.2 per cent, within a period of ten hours during the day. In a later report Rabinowitch and Strean[2] concluded from further studies that hemoglobin is distributed on the surface of the cell stroma and that corpuscular amount of hemoglobin is proportionate to the cell surface.

Barcroft and coworkers[3] presented data to indicate that the spleen may act as a reservoir for red cells from which the circulating cells may receive additional recruits to meet certain exigencies. Osgood and Haskins[4] refer to spontaneous diurnal variations of red cells and hemoglobin but feel that further work should be done on this subject.

We are presenting herewith some carefully conducted observations† bearing on diurnal fluctuations in normal individuals. Those studied were going about their daily duties which for the most part were of a clerical nature. A few studies were made on the effect of sudden active exercise. Results are reported in Tables II and III. No attempt has been made to review the numerous opinions bearing on this subject. This has been exhaustively done by Smith[5] to whose article the reader is referred.

TECHNIC

Duplicate counts were made on each specimen with the use of a separate pipette for each count. Similar duplicate pipettings were also used for the hemoglobin determinations. The erythrocyte pipettes were shaken in a mechanical shaker for several minutes to insure an even distribution of the cells. A Bausch and Lomb modified Bürker-Newbauer counting chamber was used and care was taken to obtain an even distribution of cells over its surface when a drop was applied. Technic was carried out by a highly competent and experienced technician with the exception of the earlier observations which were made by the author. It was felt that although both workers usually agree closely in hemoglobin readings and blood counts, the results would be more comparable in studying fluctuations if each series of observations were made by a single individual.

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†Complete tables of all experiments will appear in the author's reprints.

Blood for counts and hemoglobin estimations were taken by venipuncture, care being taken to avoid undue or prolonged stasis.

As a check upon the accuracy of the technic, blood specimens were also drawn from the veins of four individuals into dry Luer syringes and discharged immediately into bottles containing dry powdered oxalate; these were thoroughly shaken and submitted by number for erythrocyte counts and hemoglobin determinations. The technician had no knowledge of the identity of the specimens thus submitted. The results which were assembled later are shown in Table I. It will be seen from Table I that although the maximal variation in cell counts was 700,000 and in hemoglobin was 7 per cent, in general the average differences between average counts and hemoglobin readings were less than the findings in the study of diurnal variations. We feel justified, therefore, in believing that the diurnal variations noted in Table II are much greater than can be explained on the basis of technical error.

DISCUSSION

In the study of diurnal variations changes of from 1 to 17 per cent of hemoglobin and from 70,000 to 940,000 erythrocytes were observed within a period of eight hours with the subject at a moderate amount of physical activity; and variations of as much as 10 per cent of hemoglobin and 900,000 erythrocytes were observed within a few minutes following active exercise, such as stair running (see Table III). Erythrocytes and hemoglobin concentrations did not always parallel each other. This observation suggests the possibility that the hemoglobin content of the individual cell is not a fixed quantity but is subject to sudden changes, or that in responding to the demands of the organism, erythrocytes with different increments of hemoglobin are added to the circulation from some depot, such as the spleen[3] with sufficient rapidity to modify materially the quantities present in the peripheral circulation.

Krumbhaar[6] states that he has always felt that "a very annoying lack of correspondence between hemoglobin and red cell counts must be largely or entirely explained by the unavoidable margins of error in the technical method." He further states that this has bothered him in more than twenty years of anemia studies, and that he occasionally notes in the literature where others have attributed false significance to the apparently uncontrollable variations.

Smith[5] although admitting in her article that "some believe in a constant number (of red cells) and some in a widely varying one" gives as the result of her own extensive observations, the opinion that "within short periods of time, certainly the eight-hour periods investigated, the total red blood cell count is practically constant, and that any fluctuations present are within the limits of error of the method." She states further that "variations in hemoglobin determinations are similar and parallel to those of the total red counts," although "large diurnal variations have been found more frequently than small ones by previous investigators."

TABLE I

SHOWING RESULTS OF ERYTHROCYTE COUNTS AND HEMOGLOBIN READINGS ON DIVIDED SPECIMENS. FINDINGS IN EACH CASE SHOULD AGREE AND VARIATIONS THEREFORE REPRESENT TECHNICAL ERROR

CASE		ERYTHROCYTE COUNTS				HEMOGLOBIN READINGS					
				MAXIMAL VARIATIONS				MAXIMAL VARIATIONS			
		TIMES BLOOD SPECIMEN DIVIDED	AVERAGE OF BLOOD COUNTS	IN INDIVIDUAL BLOOD COUNTS	IN AVERAGE OF DUPLICATE COUNTS	TIMES BLOOD SPECIMEN DIVIDED	AVERAGE OF HEMOGLOBIN READINGS		IN INDIVIDUAL HEMOGLOBIN READINGS		IN AVERAGE OF DUPLICATE READINGS	
			mil.	mil.	mil.		gm.	per cent	gm.	per cent	gm.	per cent
M. F. K.	6/18/34	4	4.740	0.290	0.215	4	12.775	93.25	0.5	4	0.35	2.5
A. R. G.	6/18/34	5	5.034	0.380	0.265	5	14.19	90.0	0.5	2	0.35	2.0
S. M.	6/25/34	5	4.980	0.280	0.195	10	14.17	89.85	1.3	7	0.8	5.0
F. P. D.	6/25/34	5	4.865	0.700	0.540	10	14.07	89.20	1.0	6	0.5	3.5

In the light of the present studies and previous observations, we do not believe that the lack of correspondence mentioned by Krumbhaar can be explained entirely on the basis of technical error. The independent fluctuations of red cells and hemoglobin over short periods of time are too great to be explained on this basis. We believe a better tentative explanation can be found in the work of Barcroft, or in the possible supposition that free hemoglobin may be taken up as such directly by the erythrocytes from certain depots, perhaps within the reticulo-endothelial system of the spleen or liver.

A few observations were made on the viscosity of blood before and after stair running by the method of Hess. With the Hess viscosimeter the rate of flow of freshly drawn blood through capillary tubes is compared with that of

TABLE II

SHOWING DIURNAL VARIATIONS IN CONCENTRATIONS OF RED CELLS AND HEMOGLOBIN, SUBJECTS ENGAGED IN SEDENTARY DUTIES. SPECIMENS TAKEN ON EACH DAY DURING WORKING HOURS AT APPROXIMATELY 9:00, 11:00, 1:00, 3:00, AND 5:00 O'CLOCK

CASE	SEX	AGE	DATE	ERYTHROCYTE COUNTS				HEMOGLOBIN READINGS			
				AVERAGE OF ALL COUNTS	COLOR INDEX	MAXIMAL DIURNAL VARIATION		AVERAGE OF ALL READINGS		MAXIMAL DIURNAL VARIATION	
						RED CELLS	COLOR INDEX				
				mil.		mil.		gm.	%	gm.	%
A. R.	F	27	6/12/33	4.28	1.02	0.51	0.19	13.2	97	1.0	8
			6/20/33	4.28	1.00	0.07	0.04	13.1	95	0.7	5
			6/28/33	4.32	0.96	0.50	0.07	12.6	92	2.3	17
M. F. K.	F	42	6/14/33	5.08	0.96	0.66	0.09	14.8	109	0.7	5
			6/21/33	4.97	0.91	0.34	0.06	13.8	101	1.3	10
			6/27/33	4.93	0.98	0.39	0.05	14.7	107	1.0	7
F. P. D.	M	25	5/ 3/33	4.64	0.96	0.60	0.07	13.8	88	1.2	7
			5/10/33	4.39	0.96	0.31	0.08	13.4	85	0.7	5
			5/17/33	4.49	0.95	0.30	0.05	13.4	85	1.5	10
B. R.	M	18	5/19/33	5.03	0.90	0.42	0.05	14.2	90	1.8	11
			5/26/33	4.84	0.88	0.10	0.09	13.4	85	1.0	7
			6/ 2/33	4.54	0.92	0.43	0.07	13.2	84	1.5	10
J. J. S.	M	28	10/10/24	4.82	0.93	0.50	0.08	13.9	88	0.5	3
E. H.	F	32	10/ 7/24	4.29	0.88	0.94	0.17	11.4	83	0.4	3
			10/10/24	4.36	0.88	0.43	0.08	11.6	85	0.1	1
M.	F	30	9/17/24	4.72	0.89	0.63	0.13	12.8	93	0.7	5
M.	F	30	9/ 2/24	4.35	0.83	0.42	0.11	10.9	80	0.8	5
R. F.	F	41	5/24/33	4.42	1.01	0.61	0.13	13.5	99	1.2	9
			5/31/33	4.57	1.01	0.28	0.06	14.1	103	0.5	3
			6/ 7/33	4.34	1.01	0.36	0.11	13.3	98	1.4	11
A. R. G.	M	27	4/29/33	4.62	1.03	0.74	0.09	14.8	94	1.7	10
			5/ 4/33	4.92	0.95	0.42	0.19	14.6	92	1.4	9
			5/12/33	4.90	0.98	0.34	0.07	15.0	95	0.8	5
A. G.	M	25	9/28/24	3.30	1.38	0.84	0.34	13 9	88	0.5	3
H. J.	M	32	5/ 8/33	5.87	0.87	0.18	0.06	15 9	101	0.7	5
			5/15/33	5.78	0.88	0.42	0.11	15 9	101	1.2	8

distilled water when both are subjected to the same pressure. The figures express the ratio of the length of the column of water to that of the column of blood, the latter being taken as unity. Thus high figures represent high viscosity and vice versa. Viscosity of the blood is due chiefly to the concentration of the suspended elements and represents internal friction between

TABLE III

EFFECT OF EXERCISE ON RED CELLS, HEMOGLOBIN, BLOOD VISCOSITY AND CELL VOLUME

CASE	SEX	TIME	R.B.C. MILLIONS PER C.MM.	HEMO.		COLOR INDEX	VISCOSITY	CELL VOLUME %
				%*	GM.			
J. J. S.	M	Control	4.49	98	15.5	1.11	4.9	35.3
		Exercise (stair running) for one minute						
		11:00	4.82	108	17.0	1.13	5.8	40.3
		11:15	5.39	97	15.3	0.91	5.1	37.3
		11:33	5.20	94	14.8	0.90	5.1	33.8
M. M.	M	Control	3.70				4.6	37.8
		Exercise (stair running) for one minute						
		11:10	4.58				5.2	41.2
		11:25	4.22				4.7	42.0
		11:40	4.36				4.6	39.1
J.	F	Control	4 39				4.5	37.1
		Exercise (stair running) for one minute						
		11:49	3.90				4.7	38.0
		11:51	3.90				4.5	37.6
		12:21	3.63				4.4	36.6
A. W.	F	Control	4.73	82	11.2	0.78		
		Exercise (stair running) for two minutes						
			5.04	84	11.5	0.75		
J. G.	M	Control	5.41	91	14.4	0.84		
		Exercise (stair running) for two minutes						
			5.97	86	13.6	0.73		
R. P.	F	Control	4.81	84	11.5	0.79		
		Exercise (stair running) for two minutes						
			5.37	94	12.9	0.79		

*100 per cent: male 15.8 gm., female 13.7 gm., per 100 c.c.

these elements. Therefore an increase in the number of cells or of their average size will increase viscosity. It has long been known that there is an increase in the size of individual cells as a result of an increased increment of carbon dioxide and probably also of lactic acid. In a recent monograph on red blood cell diameters,[7] while discussing their diurnal variation, Cecil Price-Jones states that although gentle exercise has no special influence, violent exercise such as stair running increases the diameters. His observations show increases in the average diameter of individual cells of from 0.16 to 0.6 μ or 2.2 to 8.4 per cent. These changes he attributes to increased amounts of CO_2 and lactic acid in the blood stream, since it has long ago been shown by Gürber,[8] von Limbeck[9] and by Hamburger[10] that the addition of CO_2 to blood increased the volume of the red cells. Ryffel[11] showed also that there is a considerable increase in the lactic acid in the blood at the end of short violent exercise, but not with moderate exercise of longer duration. So it would be expected that viscosity would increase following sudden exercise, and this phenomenon was shown in the few observations made. An increase of cell volume was also shown and was to be expected.

It should be noted that in two glucose tolerance tests in which hemoglobin studies were made, there was no significant variation of the hemoglobin concentration during the two-and-one-half-hour period of each test. This would seem to indicate that blood dilution was not sufficient following the ingestion of 100 gm. of glucose in 50 per cent solution to change the hemoglobin values.

SUMMARY AND CONCLUSIONS

1. Spontaneous variations in red cell counts up to 940,000 per c.mm. and in hemoglobin values up to 17 per cent were observed in normal individuals going about their usual sedentary occupations within a period of eight hours. Less marked but sudden changes were observed in those subjected to active exercise such as stair running.

2. Hemoglobin and red cell concentrations did not always vary in the same direction at the same time. This suggests that hemoglobin either is not a fixed quantity in the individual cells but is subject to variation, or that new red cell recruits with different increments of hemoglobin are added to the blood stream from time to time with sufficient rapidity to modify former values.

3. A few viscosity and cell volume determinations were made at intervals before and after exercise. The increase in both is explained by the greater number of red cells in circulation and a probable increase in the individual cell volume as a result of the circulation of larger amounts of carbon dioxide and lactic acid.

4. In two glucose tolerance tests, hemoglobin determinations were made on the bloods over two-and-one-half-hour periods and no significant variations were found. This would tend to indicate that blood dilution was not sufficient to produce appreciable variations in hemoglobin concentrations.

5. The chief purpose of this contribution is to add further evidence to the view widely held that rapid spontaneous variations in red cells and hemoglobin can occur within short intervals and that red cell and hemoglobin values do not necessarily parallel each other.

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THE LABORATORY AS AN APPROACH TO ANEMIC THERAPY*

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CIRCULATING erythrocytes are fundamentally responsible for the clinical pictures of the anemias. In anemic states the number of these cells is generally less than low normal, and frequently they present abnormal physical and chemical characteristics. Physical variations from the normal reveal themselves in shape, size, and lack of maturity. Concerning the chemical variations, it is not possible to be explicit to the same degree, since exact knowledge of the chemical constituents of the erythrocyte is almost limited to hemoglobin. The practical result, for the present, is that the outstanding abnormal chemical finding in anemia consists in a quantitative diminution of this important component of the red cell. Variations from the normal are due either to a loss of red cells or to a quantitative or qualitative failure of erythrogenesis. The loss of blood may result from a vascular leak or may be accounted for by an accelerated intravascular erythrocytic destruction. Unsatisfactory erythrogenesis may be consequent upon a number of unfavorable circumstances which influence erythropoiesis and the delivery of mature red cells to the peripheral circulation. Among these are certain congenital, nutritional and unknown factor deficiencies, the traumatism of chemical and bacterial poisons and the compression effects of neoplasms and excessive leucocytic proliferation in the leucoses.

While all of this is appreciated, we continue to meet the general assumption that the classification of anemias as primary and secondary is dependent upon the existence of a known or unknown cause and perhaps upon the type of color index. All the while, however, it is evident that a primary anemia may have a known cause and that an anemia regarded as secondary may have a wholly unknown cause. In this connection, reference can be made to the primary type of anemia observed in some patients with *Dibothriocephalus latus* infestation and to the secondary type of simple hypochromic anemia with no evident cause. As it develops, all anemias are secondary and any of the etiologic factors mentioned heretofore, whether wholly understood or not, may be responsible for an anemia. The same apparent cause, moreover, and this is more important, may be responsible for different varieties of anemias. These various types are defined by the number per unit volume of blood, the size and the pigment content of red cells so far as each of these attributes deviates from the normal, or so far as they vary from the normal in quantitative relationship to each other. On such a basis, we find that a single etiologic factor, pregnancy for example, may account for varied types of anemia. That an individual is anemic may be more

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apparent than real; decisive determination can be made only by virtue of laboratory procedures. These can, at the same time, be utilized to place an anemia among the several varieties.

It has not been many years since we were content, in our patient study, with an enumeration of red and white cells per cubic millimeter of blood, some rather unreliable estimations of its hemoglobin content and perhaps a differential leucocytic count. It is still of major importance to count the erythrocytes of suspected anemics. It is of equal importance to estimate the hemoglobin content of the blood. Parenthetically, it is preferable to make such determinations as grams per 10 c.c. of blood rather than percentage of the supposed normal. It must, however, from what has been said, be evident that determinations of the size of the red cells are needed before one can have an intelligent idea of the nature of an anemia and, more important, before one can apply a rational therapy. Erythrocytic size can be determined from a Price-Jones curve which visualizes the diameters and anisocytotic range of several hundred red cells, or from a hematocritic estimation of cell volume. Haden has recently reemphasized the value of the latter method, giving original credit to Capps. Haden makes use of an ordinary accurately calibrated centrifuge tube instead of an especially made hematocrit. This method seems easier and less time consuming; it may, moreover, detect changes which are not made evident by the Price-Jones technic.

For the purpose of classifying anemias these reports are utilized to calculate corpuscular constants or indices. The former include estimations of mean corpuscular volume, hemoglobin, and hemoglobin concentration, expressed as absolute figures. These are used by way of comparison with figures established by examination of a series of normals. The indices are calculated on a basis of comparison with ideal normals. Thus the volume index is expressed by volume per cent over red cell percentage when five million is counted as a normal number of cells and 46 is the volume coefficient, or the number of cubic centimeters of packed cells per five million cells per hundred cubic centimeters of blood. The normal limits of the volume index are 0.90 to 1.10. The color index is expressed as the result of dividing the hemoglobin percentage by the red cell percentage. Here, too, 100 per cent is counted as the ideal hemoglobin content and five million as the normal red cell count. The normal limits of color index are 0.90 to 1.10. The saturation index is determined by dividing the per cent of hemoglobin by the volume per cent. The normal limits are from 0.90 to 1.10. The indices of volume and saturation are the more valuable for practical purposes although the color index has been popular for many years. It is quite true that the constant figures may be more accurate as working guides, as pointed out by Clarke at the Philadelphia General Hospital, but the determination of indices seems a bit more convenient since it links present methods with those of the past. In any event, if the determination of indices leaves one in doubt, there is the possibility of resorting to a comparative consideration of the corpuscular constant values. From studies of anemic blood in this fashion, it has been demonstrated that red cells may be excessive,

normal or decreased in number, large, normal, or small in size and of increased, normal or diminished hemoglobin content. With three variables, one may have variable combinations and so it happens that Haden was able to point out that one may, from the laboratory point of view, have ten different types of anemia. For the present at least, as Wintrobe and others have found, only four of these types are outstanding in practice. They are all hypocythemic in type, but they differ in cellular size and in pigment content.

Of these different anemias, there is first of all the large celled or macrocytic group with a volume index greater than 1.1 or a mean corpuscular volume ranging from 95 to 160 cubic microns, a color index greater than 1.1 or a mean corpuscular hemoglobin of 30 to 52 micromicrograms and a normal saturation index or a mean corpuscular hemoglobin concentration of 31 to 38 per cent. Macrocytosis is produced from the megaloblastic level as Doan, Cunningham and Sabin put it, and it is believed that macrocytosis points very clearly to the lack of a maturation stimulus. When a patient presents a macrocytic anemia, this stimulus must be supplied by the use of an antianemic principle, found in a number of substances. We, therefore, prescribe whole liver, the fraction A, desiccated stomach, combinations of liver and stomach preparations, a diet after the directions given by Minot and Murphy in 1926 as being particularly rich in its vitamin content, or some especial preparation of Vitamin B. English hematologists are using marmite, a prepared extract of yeast flavored with celery seeds. Such antianemic preparations must certainly be potent and must be given in doses of adequate size. The laboratory indication that the dosage is adequate lies in an increase in the reticulocyte and erythrocyte count after stipulated periods of administration. During satisfactory treatment the volume index approaches the normal level. In this connection, it must be remembered that during infectious attacks, in the presence of old age, arteriosclerosis, and central nervous lesions, dosage must be doubled or even tripled over the usual amounts. In this respect antianemic therapy is similar to that of insulin. Intramuscular and intravenous methods are more efficacious in the case of patients who are gravely ill, who manifest severe complications, or in whom the gastrointestinal tract does not accept or take up antianemic substances. This group of large celled anemias, referred to in the current literature as pernicious anemia and the allied macrocytic anemias, includes, besides the Addisonian variety of anemia, some anemias occurring in sprue, in *Dibothriocephalus latus* infestation, in pregnancy, and in the macrocytic anemias of the tropics. Very occasionally, also, we find this same variety of anemia occurring after intestinal stricture and resection, with fatty chylous and pancreatogenous diarrheas, with gastrocolic fistula, celiac disease, carcinoma of the stomach, and after subtotal resection of the stomach. If the laboratory diagnosis is correct, if the antianemic preparations are potent and of sufficient dosage, and if the erythrogenic mechanism is not entirely exhausted, then the patient's blood picture will improve even though the disease is hopeless. This observation has been confirmed by satisfactory responses in the blood of patients suffering with carcinoma of the stomach.

The second group of anemias comprises those known as normocytic because their cell volume and pigment indices are normal, an evidence that the erythrocytes come from the erythroblastic level. They are only deficient in the number of cells per cubic millimeter. The size of the cells ranges between 80 and 94 cubic microns, the mean corpuscular constant is 27 to 32 micromicrograms while the corpuscular hemoglobin concentration is 33 to 38 per cent. The deficiency of cell numbers is logically supplied by blood transfusion if the anemia is responsible for serious symptoms. This type usually occurs as the result of acute hemorrhage and may also be observed in the various severe diarrheal states; it is also the variety noted in the different types of hemolytic anemia, in malaria, in the aplastic and semiaplastic anemias, in icterus gravis neonatorum and in sickle-cell anemia. When the laboratory finds a severe hypocythemia with no anisocytosis, poikilocytosis, polychromasia, punctate basophilia, few reticulocytes, or normablasts, and perhaps a neutropenia and thrombopenia, then the patient is placed in an aplastic category. If the blood exhibits an increased number of reticulocytes, an increased fragility of the red cells, hyperbilirubinemia, and hyperurobiliuria, the clinician has ample evidence that the anemia is of an hemolytic variety. Malarial forms and sickled cells are other evidences which the laboratory can supply.

The third group includes those anemias with a volume index lower than normal and a color index comparably low. The cells are adequately filled with pigment. They range in size from 72 to 79 cubic microns, have from 22 to 26 micromicrograms of hemoglobin and present a concentration of hemoglobin of 31 to 38 per cent. This type of anemia is the variety usually found in chronic infections including typhoid, in cardiorenal disease, bronchiectasis, in lung abscess, and in septicemia. In this same category are found the anemias of malignancy uncomplicated by hemorrhage, secondary infection, or bone marrow involvement. Sometimes acholuric jaundice exhibits a macrocytosis. Unfortunately we have no direct therapeutic answer for this group. If the cause is evident and if it is treatable, then one should obviously attack the cause. If the laboratory findings place an anemia in this group, it becomes evident that little is to be expected from the usual antianemic measures.

The fourth and last group includes those patients with anemias whose indices are unusually low. The cells measure from 50 to 71 cubic microns, they contain only from 14 to 21 micromicrograms of hemoglobin, and the hemoglobin concentration ranges from 24 per cent to 29 per cent. This group is styled hypochromic microcytic because the cells are poorly saturated with pigment and small in size. Chronic hemorrhage from any source will eventually produce this picture. The same type is noted in a number of anemic states variously known as chlorosis, simple hypochromic and achlorhydric anemias. These varieties at times are only moderately microcytic. During pregnancy one may find a state of hypochromia as the dominant deviation. Myxedema, scurvy, beriberi, pellagra, hookworm infestation, poor nutritional states in infants, gastrointestinal resection and stricture, and the several grave diarrheal states are some of the conditions in which we may find this combination of microcytic hypochromia. This is the type of anemia, lacking acutely in pigment content,

that has responded well in the past, after the exhibition of iron, and that responds even more satisfactorily today because of the increased doses of iron used. Copper in all likelihood contributes considerably to the relief of patients in this group, although no particular attention needs to be paid to the deficiency because sufficient copper will be available in the usual iron preparations and in an adequate diet. Vitamin C and thyroxin are also needed at appropriate times by patients who present this variety of anemia. In this instance, also, the secondary anemia fraction found in liver when combined with iron, will produce gratifying results. The collateral use of large doses of dilute hydrochloric acid will undoubtedly hasten recovery for those who are afflicted with this sort of anemia and who are achlorhydric. The absence of hydrochloric acid and the gastric enzymes is established by the laboratory.

In addition to the establishment of all the foregoing facts and factors through laboratory procedure, it is the laboratory to which the physician must look for assistance in making a decision as to whether or not his patient is the victim of a leucosis, whether he has an intestinal infestation, whether there is an achylia or achlorhydria, what type of diarrhea may be present, whether there is a septicemia or bacteremia, whether there is a granulopenic or thrombopenic state. In doubtful cases the pathologist in the laboratory may illumine a clouded situation by examination of the bone marrow of sternal biopsies, to say nothing of the aid which may come from the roentgenologic laboratory.

It is not to be inferred, however, that laboratory investigation in the anemias precludes all other studies. It is still good practice to obtain thorough histories, and to examine the patient well before outlining logical laboratory investigation. From the many disease entities mentioned in this presentation, it is evident that anemia occurs under many and varied circumstances. Much more than laboratory reports must be had before complete and intelligent diagnoses can be made and before comprehensive and effective therapy can be applied. Exact diagnoses can, after all, here as elsewhere, result only after every bit of evidence is obtained by *all* the means at the command of the physician; and treatment cannot be directed from the laboratory.

It may, at this point, occur to the reader that the approach outlined here is, after all, in accord with usual practice, and that it poses no new doctrine. This may be substantially true. It is hoped, however, that this presentation does clarify certain factors in our present tendencies, and emphasizes certain dangers due to attempts to experiment in several directions at the same time. It is not unusual, for instance, to find all three of the major antianemic attacks being applied at once. This is unscientific and certainly, to say the least, uneconomical. It also makes for difficulty in gauging dosage and in evaluating results. In other forms of therapy, we deprecate shotgun prescription, why not in antianemic therapy? It is not unusual also to find an assumption that the patient has pernicious anemia governing the use of liver or ventriculin therapy. No results are forthcoming and therapeutic skepticism is immediately created. Perhaps a careful analysis of the indices might show a lack of pigment saturation and a need for iron instead of liver, or perhaps the analysis

might reveal anemia requiring other methods. It should be said, in passing, that the almost universal use of liver or other antipernicious anemia preparations for all types of anemia has unfavorably influenced the cost of treatment for that group that specifically needs this.

In Conclusion.—While exclusive reliance is not to be placed upon the laboratory in the study of anemic patients and in the control of their treatment, we cannot intelligently forego the measurable directional aid that will be available if certain laboratory studies are called for in intelligent fashion, and then evaluated in the light of clinical judgment and experience. Patients should not be treated uniformly. If we approach *by way of the laboratory, in part at least,* the exceedingly difficult problems presented in the diagnosis and treatment of the anemias, the attack will be direct, the control intelligent and the therapeutic results as good as can be expected.

THE EFFECT OF JAUNDICE ON INTRADERMALLY INJECTED SALT SOLUTION*

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SINCE the intracutaneous salt solution test was described by McClure and Aldrich in 1923,[1] it has been studied in a variety of conditions with interesting results. Normally, the wheal produced by injecting 0.2 c.c. of 0.85 per cent aqueous solution of sodium chloride intradermally persists for sixty minutes or more. McClure and Aldrich[2] noted rapid disappearance of the wheal in edema, the disappearance time being roughly in inverse ratio to the degree of edema at the site of injection. Similar reduction of the disappearance time of the intradermally injected salt solution was subsequently noted in acute infections as scarlet fever and diphtheria,[3] lobar pneumonia,[4] toxemias of pregnancy,[5] areas of deficient circulation,[6] serum sickness,[7] and thyrotoxicosis.[8] Prolongation of the disappearance time was found by Thompson[9] in myxedema. White and Irvine Jones[10] have shown that the disappearance time is not affected by the hydrogen ion concentration, the osmotic pressure, or the salt balance of the injected fluid. Neither the blood flow through the injected part nor the immersion of the injected extremity in hot or cold water has any notable effect. The disappearance time is also not influenced by the concentration of the nonprotein nitrogen, cholesterol, or sodium chloride in the blood plasma. It is, however, definitely diminished by injecting minute amounts of adrenalin with the salt solution or by the direct application of cold to the local area of injection.

In the course of some other work, we noted rapid disappearance of the saline wheal in cases of jaundice. Investigating the matter further we studied

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carefully fifteen cases of well-marked jaundice (of hepatic origin). The series included eleven women and four men; the youngest was twenty-nine years of age, the oldest sixty-five years. The nature of the lesion in each case is given in Table I. The test was performed according to the original technic of McClure and Aldrich, the wheals being made on the flexor surface of the right forearm below the cubital fossa, and the end-point determined by palpation.

TABLE I

TABLE SHOWING COMPLETE PROTOCOL

NAME	AGE	SEX	LESION	DISAPPEARANCE TIME (MINUTES)	ICTERUS INDEX
1. G. W.	50	F.	Syphilitic cirrhosis of liver	(1) 30 (2) 18 (3) 6	(1) 27 (2) 34 (3) 40
2. M. G.	65	M.	Carcinoma of head of pancreas	(1) 28 (2) 22 (3) 7	(1) 42 (2) 44 (3) 60
3. F. L.	53	M.	Carcinoma of common bile duct	(1) 18 (2) 12 (3) 4	(1) 62 (2) 75 (3) 112
4. M. W.*	54	F.	Choledocholithiasis	(1) 32 (4) 70 (2) 32 (5) 64 (3) 48 (6) 80	(1) 48 (4) 11 (2) 42 (5) 7 (3) 24
5. C. L.*	39	F.	Choledocholithiasis	(1) 24 (4) 65 (2) 37 (5) 68 (3) 48 (6) 78	(1) 60 (4) 12 (2) 42 (5) 9 (3) 30
6. K. A.*	50	F.	Choledocholithiasis	(1) 38 (4) 75 (2) 30 (5) 84 (3) 52 (6) 78	(1) 52 (4) 18 (2) 26 (5) 8 (3) 22
7. I. M.	29	F.	Acute catarrhal jaundice	(1) 46 (4) 58 (2) 34 (5) 80 (3) 22	(1) 64 (3) 22 (2) 90 (4) 10
8. C. S.	49	F.	Toxic hepatitis (drug poisoning)	(1) 22 (4) 14 (2) 17 (5) 10 (3) 14	(1) 72 (3) 70 (2) 64 (4) 84
9. A. M.	58	F.	Carcinoma of head of pancreas	(1) 35 (4) 40 (2) 37 (5) 36 (3) 35	(1) 30 (3) 48 (2) 36 (4) 54
10. A. H.	58	F.	Carcinoma of head of pancreas	(1) 40 (4) 22 (2) 31 (5) 56 (3) 26	(1) 18 (3) 36 (2) 30 (4) 44
11. O. T.*	52	F.	Choledocholithiasis	(1) 38 (4) 40 (2) 30 (5) 56 (3) 24 (6) 70 (7) 72	(1) 30 (4) 30 (2) 36 (5) 18 (3) 44 (6) 12
12. M. D.*	49	F.	Carcinoma of head of pancreas	(1) 28 (4) 34 (2) 15 (5) 54 (3) 30 (6) 66 (7) 62	(1) 70 (3) 24 (2) 38 (4) 14
13. M. C.*	45	F.	Choledocholithiasis	(1) 34 (3) 65 (2) 45 (4) 68	(1) 38 (2) 18 (3) 10
14. W. S.	67	M.	Carcinoma of rectum with hepatic metastases	(1) 44 (2) 28 (3) 20	(1) 22 (2) 40 (3) 76
15. I. M.*	50	M.	Choledocholithiasis	(1) 30 (3) 56 (2) 32 (4) 72	(1) 46 (2) 20 (3) 12

*The asterisk indicates that some operative procedure was carried out designed to correct the pathologic condition and diminish the jaundice.

The disappearance time of the wheal (in minutes) as given in the table represents the average of many tests performed at intervals, and checked up by determinations of the icterus index. A control series of injections was made in another group of patients with gallbladder and hepatic disease without jaundice. Care was taken to exclude from this study any patient exhibiting fever and cardiac or renal damage.

In fourteen of the fifteen cases studied it was found that as the jaundice increased the disappearance time of the saline wheal decreased, and when the jaundice began to diminish, the disappearance time became prolonged and approached or reached the normal. In only one case (Case 9, Cook County Hospital No. 1,309,634) was there no demonstrable change. (No explanation for this is offered at present but the suggestion is ventured that there may have been irreversible chemical changes in the cells because of the jaundice.)

The exact mechanism of this interesting test is not entirely clear. Guggenheimer and Hirsch[11] offered a mechanical hypothesis to explain the rapid absorption of salt solution by edematous tissues. Cohen[12] and his colleagues believe that the mechanism of reaction is that of an increased affinity of the tissues for the water, based largely on the work of Fischer[13] who postulated that the normal turgor of a tissue depends on the physical state of its colloids and that edema results whenever the water-holding power of its colloids is increased and when a supply of free water is available. It is possible that the profound physicochemical changes occasioned by jaundice injure the cells in such a way as to alter their permeability or their chemical reaction. Belicoff[14] calls attention to the fact that the skin is an active organ, capable of reacting to various stimuli, and able to bring into play all available defensive mechanisms. Nadel's[15] work seems to show that the resorption of intracutaneous physiologic salt solution depends on several factors: the state of the peripheral blood vessels, the vegetative nervous system, elasticity of the skin, temperature, degree of intoxication, age, etc. The chemical changes in the skin may not be without some significance. The shortening of the disappearance time, therefore, cannot be looked upon as entirely due to tissue thirst. Adlersberg and Perutz[16] have even produced some evidence of a centrally acting mechanism.

SUMMARY

The intracutaneous salt solution test was studied in fifteen cases of jaundice (hepatic). In fourteen of the fifteen cases there was a definite relationship between the severity of the jaundice and the disappearance time of the saline wheal. With increasing jaundice the disappearance time of the saline wheal decreased; with diminution in the severity of the jaundice the disappearance time of wheal became prolonged and approached or reached the normal.

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THE DIFFERENTIAL COUNT AT HIGH ALTITUDES*

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In 1933, Stammers[1] reported a study of the polymorphonuclear-lymphocytic ratio at an altitude of 5,750 feet. This altitude corresponded so closely to that at Butte, Montana, that it was decided to make a corresponding study here. The official altitude of Butte (Lat. 45° 57′ N.; Long. 112° 30′ W.) is 5,755 feet, although it varies from 5,527 feet to about 6,400 feet.

Stammers found averages of 54.20 per cent polymorphonuclears and 39.72 per cent lymphocytes in his study of blood pictures in 171 healthy young adults. This percentage of polymorphonuclears was 14 per cent below, and that of lymphocytes approximately the same amount above the respective averages of 68.2 per cent polymorphonuclears and 25.8 per cent lymphocytes which he found in twelve textbooks. These averages were presumably given for areas near or at sea level. Almost all the textbooks which we use give percentages slightly lower than 68.2 per cent for the polymorphonuclears, and also give the figures in round numbers, such as "60-70 per cent," "about 65 per cent," and "62-64 per cent." Earhart,[2] quoting Schilling, gives the normal range for polymorphonuclears as 54 to 72 per cent, the average being 67 per cent. Together they average about 65 per cent.

Clark's[3] experiments and survey of the literature show that ultraviolet light stimulates a relative lymphocytosis in both man and animals. People living in relatively higher altitudes would naturally be exposed to more ultraviolet radiation from the sun than those living at lower altitudes, and this fact is given as the most plausible explanation for relative lymphocytosis at these altitudes.

Johannesburg, Africa (Lat. 26° 11′0), where Stammers made his study is much closer to the equator than is Butte. It also receives a greater percentage of sunlight, 73 per cent, compared with 57 per cent for Butte;† Switzerland 40 per cent; and London 29 per cent. The logical conclusion from these facts is that it receives even more ultraviolet radiation than Butte.

The following study was made on young healthy adults whose ages averaged 24.3 years, and most of whom had lived at this altitude all their lives, though a few had been here for shorter periods. They were nearly all prospective blood donors or students, and all were questioned about illnesses, especially colds. As said before, the study was primarily undertaken to determine the polymorphonuclear-lymphocytic ratio, but a complete blood count was done

*From the Murray Clinic, Butte, Mont.
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†Figure for Helena, Mont., north of Butte.

on all subjects to exclude any possibility of deviation due to unexpected variation in hemoglobin, red or white cell counts. Only one subject was excluded and that was a woman who showed a marked secondary anemia, though it was later seen that her polymorphonuclear ratio was practically the same as the average of the whole group. Thirteen showed unexplained white counts over ten thousand, from 10,500 to 15,100, but the average polymorphonuclear ratio of these was only about 2 per cent higher than the total of the whole group.

Alvarez[4] has found that at sea level a barometric low produces a temporary leucocytosis. The barometer at Butte usually hovers around 618 mm. of mercury, with variations to approximate limits of 600 mm. and 625 mm. The deduction from these facts could be made that the low pressure increased the total number of leucocytes, but so-called normal variations of the total white counts are probably higher than usually given. Experiments at the Washington Square College of New York University[5] revealed white counts ranging from 4,700 to 11,500 in persons at rest, and that mild activity produced no constant effect on the count. In our study, the low and high counts were 4,050 and 15,100, respectively, the average on all subjects being 8,260.

The hemoglobin readings were done with the Dare, and were read and reread by two persons familiar with that instrument. The red and white counts were thoroughly mixed by automatic shaker and by hand before being counted. Smears were stained with Wright's stain and 300 cells were counted from all portions of the slide unless the distribution was uneven, when more were counted.

One hundred subjects were studied, 25 women and 75 men. The following tables of hemoglobins, red and white counts are not from a large enough series to permit of definite conclusions but are of interest in addition to the main study.

TABLE I

	HEMOGLOBIN (DARE)	ERYTHROCYTES	LEUCOCYTES
Average of all (100)	85.88%	5,037,600	8,260
Average of men (75)	86.78%	5,172,000	8,350
Average of women (25)	83.16%	4,636,000	7,980

TABLE II

THE RESULTS OF THE DIFFERENTIAL COUNTS

CELLS	RANGE	MEAN
Polymorphonuclears	35.5-72.5	54.36
Lymphocytes	19.0-53.0	36.26
Monocytes	2.5-15.0	6.33
Eosinophiles	0.0- 6.5	2.00
Basophiles	0.0- 2.0	0.645

In comparison to this, Stammers found a mean of 54.20 per cent for polymorphonuclears.

Sachs,[6] working in Transvaal at an altitude of 4,392 feet above sea level, found a mean of 54.70 per cent in 81 individuals, so that these three different results are within 0.5 point of each other.

Ruppanner[8] examined the bloods of healthy adults at altitudes of 1,750 meters (5,741 feet) and 2,250 meters (7,382 feet) above sea level in the Swiss mountains with the following results:

1. For eight native men, altitude 1,750 m. (5,741 feet) the average leucocytic formula was as follows:

Total leucocytes 5,775

Neutrophile	49.85%
Lymphocyte	38.25%
Large mononuclears and transitional forms	8.85%
Eosinophile	2.75%
Mast cells	0.3 %

2. In twelve native women, altitude 1,750 m. (5,741 feet)

Total leucocytes 7,250

Neutrophile	59.00%
Lymphocyte	31.4 %
Large mononuclears and transitional forms	7.35%
Eosinophile	1.85%
Mast cells	0.4 %

3. In thirty-nine men visiting for a period of from several weeks to a year, altitude 1,750 m. (5,741 feet)

Total leucocytes 6,740

Neutrophile	58.75%
Lymphocyte	29.2 %
Large mononuclears and transitional forms	8.6 %
Eosinophile	2.95%
Mast cells	0.5 %

4. Visiting women, altitude 1,750 m. (5,741 feet)

Total leucocytes 7,680

Neutrophile	60.0 %
Lymphocyte	30.75%
Large mononuclears and transitional forms	7.0 %
Eosinophile	1.6 %
Mast cells	0.3 %

5. Twelve men, altitude 2,250 m. (7,382 feet)

Total leucocytes 7,670

Neutrophile	50.2 %
Lymphocyte	36.6 %
Large mononuclears and transitional forms	9.0 %
Eosinophile	4.0 %
Mast cells	0.2 %

After examining a group of healthy subjects immediately after and for some time after their arrival in the mountains, Ruppanner states that the evidence indicated that the normal leucocytic formula at high altitudes shows a normal or slightly lower total number of leucocytes, neutropenia, lymphocytosis, and a slight degree of mononucleosis.

Hartman[9] studied the bloods of members of the German expedition to the Himalayas in 1931 and gives the results as shown in Table III.

He observed signs of regeneration more marked in the red cells at high altitudes than at low altitudes.

TABLE III

TYPES OF CELLS	ELEVATION IN METERS							
	0	2800 (9184) FT.	4400 (14,432) FT.	5600 (18,368) FT.	6300 (20,664) FT.	6600 (21,648) FT.	7200 (23,616) FT.	7600 (24,928) FT.	600 (1968) FT.
Eosinophile	3%	5%	2%	3%	3%	3%	3%	2%	3%
Rod-shaped nucleus	1	2	1	1	1	1	3	1	1
Segmented nucleus	64	44	50	54	56	58	61	63	52
Lymphocytes	29	45	42	39	37	32	27	29	40
Large mononuclears	3	4	5	3	3	6	6	5	4

An unrecalled reference stated that the eosinophiles increased in percentage toward summer as the sun's rays became stronger. It happened that this work was done in the winter of 1933-1934 and the spring of 1934. The two seasons were arbitrarily divided in March and the first 57 taken in the winter showed an average of 1.87 per cent eosinophiles; the last 42 taken in the spring showed an average of 2.21 per cent eosinophiles. These figures would tend to bear out the findings of the writer whom we cannot recall.

SUMMARY

1. Our findings agree with those of other writers that a relative lymphocytosis occurs at high altitudes.

2. This means a decrease of from 10 to 14 per cent in the polymorphonuclears in contrast to what might be expected at sea level, which fact must be taken into consideration when making a diagnosis or prognosis.

3. A slight increase (0.34 per cent) in eosinophiles was found in the counts taken during the spring months as compared to those taken during the winter months.

4. Thirteen per cent of the group of 100 healthy young persons are found to have a total leucocytic count over the usually accepted upper normal limit of 10,000.

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ARE BRUCELLA ABORTUS AGGLUTININS IN THE BLOOD STREAM PRODUCED BY ACTIVE OR PASSIVE IMMUNIZATION?*

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INTRODUCTION AND HISTORICAL

THIS study was undertaken in an attempt to determine whether the presence of *Brucella abortus* agglutinins in blood serum indicates active or passive immunity. When this work was started there was a question as to whether specific abortus agglutinins in human blood were of sufficient diagnostic value to determine active abortus infection when cultural findings from the blood and urine are negative. Although it was later found that *Brucella abortus* does occur in the blood stream of man, the above question still remained of academic interest, as its interpretation is of significance in cases where a high agglutination titer is obtained when the blood and urine cultures are negative.

Specific agglutinins for *Brucella abortus* in the blood stream of human beings have been reported by various investigators who were unable to determine whether their results indicated active or passive immunity. Larson and Sedgwick,[1, 2] after examining the blood sera of 425 children consuming market milk, found 17 per cent to contain antibodies against *Brucella abortus* by the agglutination and complement fixation tests. In two other groups 40 per cent and 48 per cent of the serums, respectively, were positive for *Brucella abortus*. As they had previously succeeded in infecting guinea pigs by feeding market milk known to contain *Brucella abortus*, they did not believe passive immunity so highly probable but were of the opinion that active immunity may have arisen as a result of infection. Children fed milk from a clean herd did not develop antibodies for *Brucella abortus*. Cooledge[3] fed raw milk of high antibody content to human subjects and concluded that antibodies in the blood stream of human beings are the result of passive immunity due to the absorption of antibodies from the milk and not to infection. The fact that the individuals were apparently healthy at all times during the feeding experiments indicated to him that there was no active immunity. Wilson and Nutt[4] stated that antibodies were present in a certain proportion of milk-consuming children and in a still lower proportion of adults. They were doubtful as to whether this was the result of infection or absorption of antibodies present in the milk. Carpenter and Boak[5] fed heated milk containing *Brucella abortus* and their respective agglutinins in high titer to two groups of male students. They were unable to demonstrate agglutinins in their blood

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serums and concluded that *Brucella abortus* agglutinins in human blood serum are the result of active infection. Carpenter and King[6] state in their "Symposium on Undulant Fever" that "the presence of specific abortus agglutinins is evidently a result of an active infection instead of a passive absorption of such antibodies from drinking milk containing them." They contend that undoubtedly some of the agglutinins from the milk serum are absorbed, but because of the great dilution factor due to the amount of blood in the tissues, they cannot be demonstrated.

EXPERIMENTAL

A series of experiments was undertaken in which groups of guinea pigs were fed living *Brucella abortus* cultures and *Brucella abortus* agglutinins. As recent reports of successful oral vaccination suggested that active immunity might follow the ingestion of dead bacteria, a series of tests using killed *Brucella abortus* was also included.

PROCEDURE

Healthy normal guinea pigs were placed in clean metal cages in groups of six. Each cage was supplied with from 200 c.c. to 350 c.c. of pasteurized milk daily, this not only serving as a substitute for water but also as a convenient means of administering the antigens and serum in desired amounts. Two strains of *Brucella abortus*, C M-90 isolated from certified milk and C6-3 cultured from the spleen of a guinea pig inoculated with milk from a cow infected with *Brucella abortus*, were employed in feeding. On finding these to have become unsatisfactory, strains H-1751 and H-1767 isolated from calf fetuses, were obtained from Professor Hart of the University of California. Suspensions of living bacteria were made by washing up forty-eight-hour Blake bottle cultures in 50 c.c. of sterile physiologic salt solution. Killed antigens were similarly prepared by heating for one hour at 60° C. or by adding 0.18 per cent formalin, sterility tests being carefully checked before use. Uniform dosages were insured by standardization in Hopkins vaccine tubes, 6.25 c.c. of a 1 per cent suspension or its equivalent constituting the daily dosages (approximately 10 billion bacteria).

Specific agglutinins were obtained by inoculating goats subcutaneously with 5 c.c. of killed *Brucella abortus* at weekly intervals. Titers, checked once a month, ranged from I-5,120 to I-10,240, sufficient serum being added to the milk to give the desired titer.

After each guinea pig was weighed and found to be negative for *Brucella abortus* agglutinins, feeding was commenced. Weights and titers were recorded once a month during the period of feeding. If the agglutination tests were positive in an appreciable titer, the animals were sacrificed and examined for typical lesions of *Brucella abortus* infection. Spleens showing enlargement or suspicious lesions were cultured on plain agar slants and incubated in 10 per cent carbon dioxide. Growth of organisms morphologically resembling *Brucella abortus* was serologically checked by the agglutination test. Feeding was continued for several months if the animals had developed no titers.

RESULTS

Experiment I.—Two series of guinea pigs were run concurrently using two strains of *Brucella abortus,* C6-3 and C M-90.

Series (1): Two sets of six guinea pigs each were placed in separate cages, one set being fed 6.25 c.c. of a 1 per cent suspension of living *Brucella abortus* C6-3 in 350 c.c. of milk, and the other 350 c.c. of milk only.

Daily feeding was discontinued thirteen days later upon finding this strain of *Brucella abortus* to have become avirulent and overgrown with gram-positive sporulating rods. A fresh antigen was prepared from strain H-1751. The animals were rechecked for abortus agglutinins and on being found negative, feeding was resumed again in five weeks.

One month later titrations were made. The animals fed living culture showed titers ranging from I-320 to I-1280. Autopsies revealed swollen, mottled spleens ranging from one and one-half to four times normal in size. Cultures showed pure growth of *Brucella abortus.* The controls showed no agglutinins at this time, one having died from an undetermined cause. Three and one-half months later, three controls gave titers of I-10 and cultures of the spleens of two of these showed growth of *Brucella abortus.** Cultures made from the spleen of the remaining pig and the two others which developed no agglutinins, were negative after prolonged incubation, although one showed growth of a streptococcus.

Series (2A): Two sets of six guinea pigs each were fed 350 c.c. of milk containing 6.25 c.c. of a 1 per cent suspension of living *Brucella abortus* C M-90 and 350 c.c. of milk, respectively. After thirteen days, feeding was discontinued as this strain of *Brucella abortus* grew very sparsely making it difficult to prepare sufficiently large amounts of antigen.

Five weeks later, before feeding was to be resumed with a new antigen, titrations were made. The living culture series was found to agglutinate *Brucella abortus* completely in a dilution of I-160. A subsequent titration sixteen days later showed titers ranging from I-640 to I-10,240. Autopsy showed spleens in five guinea pigs which were mottled and enlarged from one and one-half to three times. All gave pure cultures of *Brucella abortus.* The controls had developed no agglutinins. Spleens of all controls were normal in appearance and cultures were negative for *Brucella abortus.*

Series (2B): The experiment was resumed by feeding six guinea pigs 6.25 c.c. of a 1 per cent suspension of living *Brucella abortus* H-1767 in 350 c.c. of milk, and another six animals 350 c.c. of milk only.

In twenty-one days, the guinea pigs fed living organisms had lost weight and had developed titers ranging from I-40 to I-80. Feeding was continued and five weeks later titers ranged from I-1,280 to I-10,240. At autopsy, one spleen was normal in size, the remaining five being enlarged two to four times. All were characteristically mottled and *Brucella abortus* was recovered in pure culture in each case. Of the control series, five were still living at

*Investigation determined that in daily cleaning of the cages, the feeding pans of the control series had been contaminated with small doses of *Brucella abortus,* thus accounting for the discrepancies. This was carefully controlled in all later experiments.

three and one half months, one having died previously from an unknown cause. Three gave suspicious agglutination tests in I-10 and two were negative. Autopsy findings and cultures of all were negative for *Brucella abortus*, one culture, however, showing a streptococcus contamination.

It is evident from these experiments that living *Brucella abortus* administered orally to guinea pigs results in active infection. Agglutinins in relatively high titers are developed and autopsy findings reveal typically mottled and usually enlarged spleens from which *Brucella abortus* can be successfully isolated.

Experiment II.—Twelve guinea pigs were separated into two cases of six animals each. The animals in one cage were fed 350 c.c. of milk containing *Brucella abortus* agglutinins in a titer of I-80 which is higher than any titer observed in raw milk in this laboratory, those in the other cage receiving milk only. After daily feeding over a period of seven months, no agglutinins were detected in the blood of the test series, the controls likewise being negative.

A suggestion was made by Professor Hart of the University of California that a I-80 dilution of *Brucella abortus* agglutinins might not be sufficient to induce passive immunity in guinea pigs to a degree high enough to be detected by the agglutination test. By verbal communication, he informed us that he had seen cow's milk with titers considerably higher than I-80.

Fourteen young weaned guinea pigs weighing from 150 to 200 gm. were segregated into two cages of seven animals each. Those in one cage were fed 200 c.c. of pasteurized milk and sufficient goat serum to make an agglutinin titer of I-640 which is eight times as concentrated as in the previous experiment. Those in the other cage received milk only. One of the test pigs died after two months from an unknown cause. Two control animals died within several months of streptococcus infections and one died after the first test bleeding. Titrations made at one- or two-month intervals showed no agglutinins for *Brucella abortus* in the test or control series after daily feeding over a period of five months.

These findings indicate that the daily administration of *Brucella abortus* agglutinins to guinea pigs per os over a period of several months does not result in absorption of agglutinins to such an extent as to be detected by the agglutination test.

Experiment III.—Comparatively recent studies in the field of immunology have resulted in reports by various investigators on the possibility of successful vaccination by the oral route. This subject has been reviewed by Enlows[7] and suffice it to mention that Hoffstadt and Thompson[8] have recently produced agglutinins in the blood serums of human subjects by oral inoculation of triple typhoid vaccine. They found no delay in the appearance of agglutinins over that of the subcutaneous method. Bile seemed to be an aid in the production of these antibodies.

Two antigens, one killed with heat and the other with formalin, were prepared with each of the following organisms: *Brucella abortus* H-1751, *B. typhosus* Rawlings, and *B. dysenteriae* Shiga #204 (American Type Collection). Sterility tests were carefully checked before use and all cages and

feeding pans were thoroughly cleaned and disinfected. After having been checked for the absence of agglutinins, six groups of six guinea pigs each were administered the above antigens in 300 c.c. milk, respectively. A seventh group of six animals, receiving milk only, served as a control.

After daily feeding for seven months, no agglutinins had been stimulated by either antigen of the organisms tested. The controls were also negative.

The effect of bile on the stimulation of agglutinins in guinea pigs following oral administration of these bacteria was studied next. On being found free from agglutinins, each animal in cages of six, was fed 2.5 c.c. of bile in licorice powder. After being deprived of food and water for twenty-four hours, they were fed formalinized antigens of *Brucella abortus*, *B. typhosus* Rawlings, and *B. dysenteriae* Shiga in 250 c.c. of milk, a control set receiving milk only.

Daily feeding was continued for one month and at the end of this period, titrations failed to detect the presence of agglutinins for *Brucella abortus*, *B. dysenteriae* Shiga, and *B. typhosus*.

These experiments indicate that in guinea pigs the administration by mouth of large doses of certain killed bacteria daily even over a period of seven months does not stimulate the production of antibodies which can be detected by the agglutination test. Preparation of the intestinal mucosa with bile also failed to stimulate the production of these antibodies.

DISCUSSION

These results show that in guinea pigs agglutinins arise following an active infection with *Brucella abortus*. Dead bacteria administered by mouth in large doses do not stimulate the production of agglutinins in guinea pigs even though the intestine is previously prepared with bile. The administration of abortus agglutinins by mouth eight times as concentrated as usually found in infected milk does not result in absorption of antibodies to a degree that can be detected by serologic tests. The development of agglutinins for *Brucella abortus* in the blood of guinea pigs only after the administration of living bacteria indicates that agglutinins are the result of an active immunity following infection.

By analogy, these experiments indicate that *Brucella abortus* agglutinins in the blood stream of human beings are undoubtedly the result of infection as first expressed by Larson and Sedgwick.[1, 2] The feeding of large amounts of high titered serum daily to guinea pigs over a period of several months with no appearance of agglutinins would seem to indicate that agglutinins in human blood also have not been absorbed through the intestinal wall from milk consumed and are, therefore, not due to passive immunity as expressed by Cooledge.[3] The stimulation of active immunity by dead bacteria which might be present in pasteurized milk seems unlikely, as the number of bacteria present in milk for human consumption certainly would not compare with the excessive doses of bacteria used in these experiments. Therefore, *Brucella abortus* agglutinins in human blood must indicate infection and active immunity, and their presence would suggest present or past infection even though urine and blood cultures are negative.

CONCLUSIONS

1. Daily feeding of specific abortus agglutinins over a period of seven months in the concentration usually found in milk did not result in the detection of agglutinins in the blood of guinea pigs. Similar feeding over a period of five months with abortus agglutinins in a concentration eight times as great failed to show the presence of these antibodies in the blood of guinea pigs.

2. Daily feeding of killed *Brucella abortus* cultures over a period of seven months did not result in the formation of agglutinins in the blood of guinea pigs. Results were also negative even though the intestinal mucosa was previously prepared with bile. Control tests with *B. typhosus* and *B. dysenteriae* Shiga showed no development of agglutinins for these organisms.

3. Daily feeding of living *Brucella abortus* cultures to guinea pigs produced an active infection which was confirmed by agglutination tests and by recovery of *Brucella abortus* in pure culture from the spleens at autopsy.

4. Our results indicate that in guinea pigs, specific abortus agglutinins are not due to passive immunity or reaction to killed abortus antigen, but are due to active infection with *Brucella abortus*.

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EOSINOPHILIA IN SYPHILIS*

A Comparative Study of the Differential Leucocyte Counts in 100 Positive and 100 Negative Blood Wassermann Cases

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THE practice of making routine differential leucocyte counts on all allergic patients and also in a majority of other new patients examined, and the finding of an eosinophilia (5 to 10 per cent and at times higher) in an occasional patient having no allergic clinical manifestations, a negative history of syphilis, and showing no syphilitic symptoms, but whose blood Wassermann test subsequently proved positive, has interested me for a number of years.

A recent connection with the Medical Service of the Philadelphia County Prison, with its vast amount of clinical material among a yearly turnover of more than 30,000 men, offered the opportunity, through the courtesy of Dr. C. Y. White, director of Philadelphia Municipal Laboratories who had the Wassermann tests made, to make a comparative study of the differential leucocyte counts in 100 men having positive and 100 men with negative blood Wassermann reactions at the Clinical Laboratory in Moyamensing Prison.

Physiologists have not given us much positive information as to the normal function of the blood leucocytes. Numerous hypotheses have been suggested following many investigations as to their function in health and under various pathologic conditions, but the significance of the altered proportional relation of the cells in the differential leucocyte count in connection with various biologic and immunologic reactions is far from being definitely understood.

With our increased knowledge the last few years of the part played by the reticulo-endothelial system in the origin of blood cells, and with more exact evidence on their biologic development we should be able by intensive and careful clinical observation to interpret more definitely the significance of the percentage range of the leucocytes in the differential count. Indeed, the differential leucocyte count seems destined to furnish some of the most important information of all the studies being made of the formed elements of the blood. The interpretation and evaluation of a study of the proportional percentage of the cells in the differential leucocyte count under various physiologic, immunologic and therapeutic conditions is undoubtedly becoming of more aid to the clinician, and it is with this fact in mind that the present report is submitted.

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BLOOD FINDINGS IN SYPHILIS

The literature on the whole is rather indefinite when one reviews the reported data on "Blood Findings in Syphilis." Certainly no typical or specific morphologic blood picture in syphilis is recognized, or has been established. Piney[1] says that the relation of syphilitic infection to "blood diseases" has often been discussed, but with very discordant results, and states that the whole subject is extremely obscure. In connection with any blood picture in a patient who has a positive Wassermann reaction, it is important of course to eliminate other diseases. When syphilis is associated with any disease it is uncertain, to say the least, that syphilis is alone the causative factor in the altered blood picture, and it is especially necessary to rule out diseases commonly recognized as allergic in positive Wassermann cases whose differential leucocyte counts show an eosinophilia.

SYPHILIS AND THE ERYTHROCYTES

A rather extensive literature is available dealing with the effect of syphilis on the red blood cells, hemoglobin, color index, etc. In 1928 Clyde L. Cummer[2] of Cleveland reported a very comprehensive review of the literature on "Anemia and Other Blood Changes in Syphilis," and recorded his own findings in 40 consecutive cases of early (primary and secondary) syphilis. He especially investigated the anemia of syphilis and summing up from his own experience and from reports in the literature he concludes: "that there is no final proof that syphilis may produce an anemia of the pernicious type; that syphilis may merely be associated with anemia and not be its actual cause; that anemia may appear at any stage of the disease and is usually of a secondary type; that in later stages of syphilis anemia may accompany visceral changes, and may be a feature of syphilitic cachexia; and that the anemia of late syphilis may be accompanied by splenomegalia, the clinical picture of which may bear a close resemblance to Banti's disease."

THE LEUCOCYTES IN SYPHILIS

From reports at hand in the literature the leucocytes in syphilitic infection seem not to have received nearly as comprehensive investigation as have the red cells. Piney[1] says "the number of leucocytes are very different in different cases." He quotes Kyrle[3] (investigation of 100 cases) who found that "there are syphilities with a normal number of leucocytes, those with a slight leucocytosis and others with a well-marked leucocytosis in about equal proportions." He reports "no relationship between the severity of the infection nor the extent of cutaneous manifestations, and the leucocytic blood picture." He states that "cases with a definite leucocytosis usually show an immediate fall to about normal as a result of treatment."

Pepper and Farley[4] report that "an increase of white cells up to 12,000, and even 20,000 is not unusual in secondary syphilis," and say "that this rise is always due to an actual lymphocytosis; the lymphocytes forming 50 per cent or even over 60 per cent of the increased total count." Stokes[5] states: "The combination of moderate secondary anemia with lymphocytosis in women always deserves a Wassermann test and a careful search for clinical signs."

In Cummer's[2] review of the literature (1928) he states that "in certain cases of syphilis with enlarged liver and spleen the degree of leucocytosis, of the large lymphatic type, is such that leucemia may be suspected," but concludes "that though syphilis may produce the blood changes of leucemia in certain cases, the picture is a simulated one as shown by necropsy observations." A lymphocytic leucocytosis in congenital syphilis is reported by many clinical observers.

Cummer further discussing the effect of syphilis on the leucocyte count quotes Hazen[6] who found that in untreated cases of syphilis there was a slight leucocytosis; that severe cases showed a higher total count and a higher relative and actual neutrophilia than do the milder cases. He felt that patients showing a high lymphocyte and low neutrophile count responded better to treatment than those showing the reverse lymphocyte-neutrophile picture. Cummer also refers to Eason's[7] report of one patient with secondary syphilis in whose blood was found "a fair number of eosinophilic and neutrophilic myelocytes." Zanca[8] under the pseudoleucemic form of congenital syphilitic anemia refers to an "eosinophilic type" which he says is rare, but is accompanied by a great increase of eosinophiles.

In the six differential leucocyte counts recorded in Cummer's series of 40 positive Wassermann cases, one patient showed a relative lymphocytosis, and another patient, complicated with an atrophic arthritis, had a 7 per cent eosinophilia in each of two counts.

I have found no report recording a series of differential leucocyte counts in syphilis, and the findings in the present series of 100 cases appear to be unique so far as it has been possible to correlate them with the available reports furnished by the literature.

METHOD OF INVESTIGATION

Differential leucocyte counts were made on 21 patients with positive Wassermanns who showed evidence of *primary lesions,* and on 9 patients who had *secondary symptoms* (skin manifestations). Then coincident with routine taking of blood for Wassermann tests, in prisoners assigned to certain work, blood smears were made until we had accumulated 70 additional differential counts (making a total of 100) on men whose blood Wassermann tests were positive and 100 differential counts on men whose blood Wassermann tests were negative. Among the 70 men with positive Wassermanns who presented no primary or secondary lesions, 23 admitted, after we had received the positive report on their blood, a syphilitic history, and that they had had previous antisyphilitic intravenous injections. Forty-seven, however, of the 70 with positive reactions were typical asymptomatic cases. They had no signs or symptoms of syphilis, denied a previous syphilitic history and did not know that their blood was positive to the Wassermann reaction. In the series of men giving positive reactions no men with a history of diseases commonly recognized as allergic were included, although we found three cases of asthma and four of urticaria among men with positive Wassermann reactions. One man having a positive reaction was also found but not included in the positive series, who had been receiving a bismuth

preparation intramuscularly and had developed a marked exfoliative dermatitis. In this patient total white counts of 26,400, 12,000, and 19,600 were recorded at weekly intervals with the differential counts showing respectively 37, 35, and 38 per cent of eosinophile cells. There was no relative or absolute lymphocytosis present in this case, the leucocytosis being of the polymorphonuclear neutrophilic type. The stools were negative for ova and parasites.

While no cases of allergy were included in accumulating the differential counts in the series of 100 men showing negative blood Wassermann reactions, as a group they cannot all be considered normal subjects, since a few had a cardiovascular or a gastrointestinal disturbance and a number presented various neurotic manifestations for which in most instances there was some physical basis. Among differential counts made on men with negative Wassermann reaction but not included in the tabulated series were 2 cases of asthma, 2 of urticaria, 1 of psoriasis, and 4 of generalized pruritus. Among the men with negative Wassermann reactions was also one man who had eosinophile counts of 20, 25, and 18 per cent, respectively, on different days over an interval of a month. Examination of his stools revealed the presence of Trichuris trichiura. The total white counts in this case were 13,600, 15,200, and 10,200, the leucocytosis being of the polymorphonuclear type.

It is realized that it would have made the investigation of more value to have included a total white count in each case and also to have counted 200 to 400 cells instead of only 100 in each differential count, but the circumstances under which the work was done made such a procedure impossible. In 14 of the men having positive reactions we were able to make total white counts which ranged from 5,200 to 12,000 but they are not considered in the analysis. We were, however, able to make eight differential counts on separate days, on each of six men with strongly positive Wassermann reactions and detailed hemograms of this smaller group are appended.

The smears for differential counts were stained with Wright's stain, and in order to eliminate the personal equation in the technical counting of the blood all counts were made by Miss Elizabeth Monaghan, a Kolmer trained technician.

THE NORMAL PERCENTAGE RANGE OF LEUCOCYTES IN THE DIFFERENTIAL COUNT

As a basis of comparison and to interpret intelligently the findings in the present investigation, it is necessary briefly to refer to the normal range of the leucocytes in the differential count. Hematologists and physiologists are not agreed in stating what may be considered as the normal percentage range of the granular leucocytes—polymorphonuclear neutrophiles, eosinophiles, and basophiles. There is rather general agreement as to the normal range of the lymphocytes and also of the large hyaline monocytes (which include the type of cell formerly designated transitional).

Piney[1] states that in health the polymorphonuclears are about 60 per cent, the eosinophiles 3 or 4 per cent, the basophiles a fraction of 1 per cent, the lymphocytes 20 to 25 per cent, and the monocytes 4 to 8 per cent. Clough[2] says the polymorphonuclears constitute 60 to 65 per cent of the leucocytes in normal blood; eosinophiles 0.5 to 3 per cent; basophiles 0.5 per cent or less;

lymphocytes 20 to 25 per cent; monocytes 6 to 8 per cent. Pepper and Farley[4] say in adult human beings, the polymorphonuclears make up from 55 to 75 per cent of the total white blood cells; eosinophiles 1 to 3 per cent; basophiles 0.5 per cent; lymphocytes 20 to 30 per cent; monocytes 5 to 10 per cent.

Howell's[10] normal range of the various types of white blood cells is wide enough to include the normal limits of most hematologists, and in analyzing and comparing the findings in the present series of differential leucocyte counts in 100 adult males with positive and 100 with negative Wassermann reactions the following normal range as given by Howell has been used: Polymorphonuclears, 60 to 75 per cent; small lymphocytes, 20 to 25 per cent; large lymphocytes, 1 per cent or less; eosinophiles, 4 per cent or under; basophiles, 1 per cent or less; transitionals or hyaline monocytes, 2 to 10 per cent.

There is general agreement that over 4 per cent of eosinophiles in the white blood count shall be regarded as an *eosinophilia*. Hematologists also generally agree there is no clinical advantage in distinguishing between the large and small lymphocytes, and that the normal range of the combined lymphocytes is 20 to 25 per cent. In the present study, therefore, when the combined small and large lymphocytes total over 25 per cent a *lymphocythemia* is regarded as being present; when the polymorphonuclears are above 75 per cent a *neutrophilia* is considered present; and since the basophiles and large monocytes occurred proportionally in so small a percentage in this series and fell well within the recognized normal range they have not been considered.

HEMATOLOGIC FINDINGS IN PRESENT STUDY

For the purpose of hematologic analysis and clinical interpretation the differential leucocyte counts in the 100 men with positive and 100 with negative Wassermann reactions in the present study are presented in the following summarized form:

In the series of 100 men with positive blood Wassermann reactions the differential leucocyte counts show:

40% with an eosinophilia	Eosinophiles,	5 to 10%
74% with a lymphocythemia	Combined lymphs,	26 to 50%
10% with a neutrophilia	Polynuclears,	76 to 89%

In the series of 100 men with negative blood Wassermann reactions the differential leucocyte counts show:

4% with an eosinophilia	Eosinophiles,	5 to 6%
68% with a lymphocythemia	Combined lymphs,	25 to 53%
16% with a neutrophilia	Polynuclears,	76 to 87%

When the "percentage range" of the cells in the differential leucocyte counts of the 100 men with positive and the 100 giving negative Wassermann reactions are compared with Howell's "normal range," as given above, several definite and interesting deductions appear. The most striking, however, is the finding that 40 of the 100 men with positive Wassermann reactions show the

presence of an *eosinophilia,* i.e., the eosinophile cells were above 4 per cent, Howell's upper limit of the normal range. Two men had 10 per cent, two 9 per cent, three 8 per cent, nine 7 per cent, eleven 6 per cent, and thirteen 5 per cent of eosinophiles in the differential counts. In only four of the men having positive reactions no eosinophiles were found in counting 100 cells per slide. In the series of 100 men with negative Wassermann reactions, however, only four showed the presence of a moderate eosinophilia. One man had 6 per cent and three had 5 per cent of eosinophile cells in the differential count, while in twenty of the men giving negative Wassermann reactions no eosinophiles were found in counting 100 cells per slide.

The finding of a lymphocytic increase above the normal range in 74 per cent of the positive cases, and of a polymorphonuclear increase above the normal range in only 10 per cent of the positive cases confirm previous reports recorded in the literature that a lymphocytosis is a characteristic finding of syphilitic infection.

CLINICAL ANALYSIS

For the consideration of a more detailed clinical analysis and comparison, the differential leucocyte counts in the 100 men with positive Wassermann reactions are separated into four clinical groups which summarized reveal the following:

Group I Includes 21 Cases With Initial Lesions:

Nine (42.85%) show an eosinophilia	Eosins,	5 to 10%.
Thirteen (61.90%) show a lymphocythemia	Lymphs,	26 to 48%.
Four (19.04%) show a neutrophilia	Polys,	76 to 85%.

Group II Includes 9 Cases With Secondary (Skin) Manifestations:

Four (44.44%) show an eosinophilia	Eosins,	5 to 6%.
Seven (77.77%) show a lymphocythemia	Lymphs,	26 to 45%.
None show a neutrophilia	Polys under	75%.

Group III Includes 47 Cases Who Never Received Treatment and Who Did Not Know That Their Blood Wassermann Reaction Was Positive:

Twenty-one (44.68%) show an eosinophilia	Eosins,	5 to 9%.
Thirty-five (74.44%) show a lymphocythemia	Lymphs,	26 to 50%.
Three (6.38%) show a neutrophilia	Polys,	76 to 83%.

Group IV Includes 23 Cases Who Had Received Previous Intravenous Treatment:

Six (26.08%) show an eosinophilia	Eosins,	5 to 7%.
Nineteen (82.60%) show a lymphocythemia	Lymphs,	26 to 40%.
Three (13.04%) show a neutrophilia	Polys,	76 to 89%.

DISCUSSION OF CLINICAL GROUP FINDINGS

The Eosinophilic Granulocytes.—In the entire series of 100 men with positive Wassermann reactions (including the 77 in various stages of syphilitic infection and the 23 who had received chemotherapeutic treatment) 40 per cent showed the presence of a blood eosinophilia. Upon closer analysis in the clinical group classification it is of interest to note in Group 1 (men with initial lesions) and in Group 2 (men with skin manifestations) that the number of men showing an eosinophilia varied but slightly, being 42.85 per cent and 44.44 per cent, respectively.

It is more interesting, however, to find in Group 3 (men who showed no symptoms of syphilis, who gave a negative syphilitic history, and who did not know their blood Wassermann was positive) that the percentage of cases showing an eosinophilia is even a fractional percentage higher, 44.68 per cent, than in patients with primary and secondary lesions (Groups 1 and 2). Then in Group 4 (men who had received intravenous therapy) we find that the number of men showing an eosinophilia drops to 26.08 per cent, slightly more than half as high as the untreated men in various stages of the disease (Groups 1, 2, and 3).

The Lymphocytes.—The combined small and large lymphocytes in the entire series of 100 men with positive Wassermann reactions were above Howell's normal range (20 to 25 per cent) in 74 per cent of the cases. In Group 1, the men showing initial lesions, 61.90 per cent showed a lymphocythemia; in Group 2, men with secondary (skin) manifestations, the number of men showing an increase of lymphocytes above the normal range was 77.77 per cent; in Group 3, men who had no symptoms and did not know they had positive blood Wassermanns, 74.44 per cent showed a lymphocythemia; while in Group 4, the men who had received previous intravenous therapy, the percentage showing a lymphocytic increase was higher than in the other three groups, 82.60 per cent.

The Polymorphonuclear Neutrophiles.—In only 10 per cent of the 100 men with positive Wassermann reactions were the polymorphonuclear cells in the differential count above Howell's upper limit of the normal range, 75 per cent. Their percentage range above normal was highest in Group 1, men with initial lesions, 19.04 per cent; while in Group 2, men with skin manifestations, in none of the men were the polys above 75 per cent in the differential count; in Group 3, men with no symptoms or signs of syphilis, only 6.38 per cent of the cases showed a poly count above the upper limit of Howell's normal range; and in Group 4, men with previous treatment, 13.04 per cent showed a neutrophilia.

RECAPITULATION

Briefly the chief findings in the series of 100 differential blood counts on 100 nonallergic men with positive Wassermann reactions are:

First, that 40 per cent showed an eosinophilia ranging from 5 to 10 per cent.

Second, that in group 3, men *without* clinical symptoms and who did not know they had positive blood Wassermanns, the percentage of men showing an eosinophilia was as high (44.68 per cent) as the men with primary lesions (42.85 per cent) and as the men with secondary lesions (44.44 per cent).

Third, that 74 per cent of the 100 men having positive reactions showed a lymphocythemia with a relative increase of the large lymphocytes.

HEMOGRAMS ON SIX MEN WITH POSITIVE (4-PLUS) WASSERMANN REACTIONS

Appreciating the daily variability of blood counts in health and disease, and realizing that our findings with only one differential count of 100 cells each in the four clinical groups totaling 100 men with positive Wassermann reactions are not unalterable conclusions, but may be regarded as only suggestive, a more

complete and intensive study was made on six men with strongly positive (plus 4) reactions, whose stools were negative for ova and parasites and who had received no previous medication. Eight differential counts were made in each case on separate days within a period of two weeks. These forty-eight counts of 100 cells each are tabulated in the modified Schilling hemograms (Tables I, II, and III).

TABLE I

HEMOGRAMS ON SIX MEN WITH POSITIVE (PLUS 4) WASSERMANN REACTIONS

CASE 1.—W. P., colored, aged thirty-five years, married, one child. Wassermann plus 4, Jan. 2 and 5, 1933.

DATE	W.B.C.	GRANULAR LEUCOCYTES					LYMPHOCYTES			MONOCYTES
		YOUNG FORMS		MATURE FORMS						
		MYELO.	BAND	POLY.	EOSIN.	BASO.	SMALL	LARGE	COMB.	(TRANS.)
1/14	6000	2	2	47	5	0	40	4	44	0
1/15		0	2	44	7	0	40	6	46	0
1/16		1	5	36	3	0	42	13	55	0
1/17		0	5	40	2	1	40	12	52	0
1/18		2	3	40	3	0	43	9	52	0
1/19	6400	0	3	42	10	0	44	1	45	0
1/22		1	1	40	12	0	44	1	45	1
1/24		0	2	40	2	0	45	11	56	0

CASE 2.—C. A., colored, aged nineteen years, single. Wassermann plus 4, Jan. 26, 1933.

2/ 3	5200	0	1	60	7	0	29	3	32	0
2/ 4		1	2	59	4	0	27	6	33	1
2/ 5		1	2	51	2	1	35	8	43	0
2/ 6		0	1	54	4	0	39	2	41	0
2/ 7		0	0	55	6	0	38	1	39	0
2/ 8		1	1	50	9	1	33	4	37	1
2/ 9	6400	0	0	62	3	0	29	6	35	0
2/10		0	0	49	5	0	34	10	44	2

TABLE II

HEMOGRAM

CASE 3.—T. E., colored, aged thirty years, married, no children. Plus 4 Kahn. Feb. 2, 1933.

DATE	W.B.C.	GRANULAR LEUCOCYTES					LYMPHOCYTES			MONOCYTES
		YOUNG FORMS		MATURE FORMS						
		MYELO.	BAND	POLY.	EOSIN.	BASO.	SMALL	LARGE	COMB.	(TRANS.)
2/ 2	8000	0	1	45	11	1	31	9	40	2
2/ 3		0	0	49	11	0	35	5	40	0
2/ 4		0	1	66	8	0	20	2	22	3
2/ 8		0	1	56	7	0	29	6	35	1
2/ 9		1	1	48	6	0	42	1	43	1
2/10		1	3	50	7	1	30	5	35	3
2/15	7400	2	5	53	6	0	29	4	33	1
3/ 3		0	1	53	13	0	31	2	33	0

CASE 4.—R. P., colored, aged forty-three years, married, one child. Plus 4 Wassermann. Jan. 8, 1933.

1/13	7000	1	3	50	1	1	31	13	44	1
1/14		0	4	61	3	0	25	7	32	0
1/15		0	1	72	2	2	19	4	23	0
1/16		4	2	42	5	0	40	7	47	0
1/17		1	2	48	2	0	42	5	47	0
1/18		3	2	48	4	1	42	0	42	0
1/19	5800	0	2	50	7	0	36	5	41	0
1/20		1	2	53	2	0	34	8	42	0

TABLE III

HEMOGRAM

CASE 5.—W. B., white, aged twenty-two years, single. Wassermann plus 4, March 30, 1934.

DATE	W.B.C.	GRANULAR LEUCOCYTES					LYMPHOCYTES			MONOCYTES
		YOUNG FORMS		MATURE FORMS						
		MYELO.	BAND	POLY.	EOSIN.	BASO.	SMALL	LARGE	COMB.	(TRANS.)
4/11		1	0	46	8	0	38	3	41	4
4/12		0	0	44	10	0	40	5	45	1
4/13		0	0	43	7	0	43	3	46	4
4/14		1	1	38	2	1	48	3	51	6
4/15		0	0	35	6	1	50	4	54	4
4/16		1	0	37	4	0	44	12	56	2
4/17		0	0	50	4	0	39	2	41	5
4/18	7400	0	0	47	9	0	37	3	40	4
CASE 6.—L. J., black, aged thirty-four years, married, one child. Wassermann plus 4, Mar. 30, 1934.										
4/11		0	0	42	4	1	46	2	48	5
4/12		0	0	35	15	0	40	4	44	6
4/13		0	0	45	13	1	35	2	37	4
4/14		0	0	56	5	0	30	5	35	4
4/15		0	0	46	8	0	34	6	40	6
4/16		2	1	43	5	0	40	5	45	4
4/17		1	0	54	10	0	27	4	31	4
4/18	6800	0	0	46	4	0	37	6	43	7

SUMMARY OF HEMOGRAM FINDINGS

The series of eight differential leucocyte counts made on each of six men with strongly positive (plus 4) blood Wassermann reactions, and tabulated in the modified Schilling hemograms, illustrates the importance of making repeated counts on separate days if the occurrence of eosinophilia is not to be overlooked. In each hemogram from two to eight of the counts showed the presence of a 5 to 15 per cent of *eosinophile* cells.

The findings in this smaller group on the whole confirm and tend to emphasize that the suggested conclusions drawn from the series of 100 positive cases may be considered on the average correct. Moreover, the counting of 100 cells on eight different days is probably of more value in substantiating the periodic occurrence of a blood eosinophilia than would have resulted from the counting of 800 cells in two or three blood smears made on the same day. It seems fair to presume, therefore, that had we made repeated differential counts, instead of only one, in the series of 100 men with positive reactions, the percentage of men showing a periodic occurrence of eosinophilia would have been much higher, possibly nearly 100 per cent.

The eight hemograms also show the uniform presence of an increase of the *lymphocytes* and correspond to a similar finding in the series of 100 cases. They confirm the reported findings in the literature of a lymphocytosis in syphilis. The increased proportional ratio of large to small lymphs is most evident in the hemogram counts and suggests a shift to the left, since the large lymphs are regarded by many hematologists as the younger form of cell.

The *neutrophilic granular polymorphonuclears* in the six hemograms are uniformly below the normal average. The younger forms of granular neutrophiles, the myelocytes and band cells, are within normal limits, so that there

is no neutrophilic nuclear shift to the left. The large hyaline *monocytes* are also within the average normal range, so that no evidence of a monocytosis was present in any of the counts.

COMMENT

*The Occurrence, Possible Function and Significance of Eosinophilia.**—In 1860 Wharton Jones[11] first distinguished finely and coarsely granular leucocytes in fresh blood. In 1878 Ehrlich[12] observed that the coarsely granular variety has special affinity for eosin stain, and since then an increased occurrence of so-called eosinophiles has been observed in a variety of conditions. Opie[13] reports the eosinophile as common to all mammalian species with an average number in man of 2 to 4 per cent. Sabin[14] has shown the percentage to vary from day to day and even from hour to hour.

Clinically eosinophiles first attracted attention and were regarded of diagnostic importance when they occurred in increased numbers in certain of the intestinal and tissue animal parasitic infections;[15] the toxins of the parasites were believed to cause disintegration of the polymorphonuclear cells with a change in staining quality of the granules. With advancing knowledge and research in hematology, an increased percentage of eosinophiles in the differential blood counts began to be reported in a large variety of conditions, including many skin diseases;[15] some of the acute exanthemas, especially scarlet fever,[15] and the postfebrile stage of measles; some forms of tuberculosis,[16] especially the intestinal variety; some cases of arthritis and rheumatism;[17] in some patients following the intravenous or intramuscular injection of the heavy metals,[15] mercury, bismuth, etc.; also following the injection of foreign proteins,[18] especially egg albumen, horse serum, and tuberculin;[19] and as shown by me[20] following intramuscular injections of venom (Crotalin) solution.

The occurrence of an eosinophilia in asthma[21] (at times to a marked degree[16]) had been noted for many years. Since about 1910, however, when Meltzer and Koessler independently first suggested the probable relationship of human hypersensitiveness and animal anaphylaxia, the presence of a greater or less degree of eosinophilia in allergic asthma has come to be regarded as a typical and a characteristic finding.

A *hypereosinophilia* occurs in some cases of Hodgkins' disease,[15] in splenomegaly, in some leucemias especially in the early stage of myelogenous leucemia, and cases of familial constitutional hypereosinophilia[22] have been reported. The occurrence of a hypereosinophilia in isolated, sporadic instances of widely different etiologic and varying pathologic conditions, is difficult to account for in our present state of knowledge, and may be regarded as more or less of a hematopoietic curiosity, interesting and worthy of mention. The constant appearance, however, of a moderate degree of eosinophilia (6 to 10 or 15 per cent) even if periodic, is a blood finding which occurs consistently enough, when searched for, in certain pathologic conditions and immunologic reactions to require an explanation.

*The writer is indebted to Dr. Huston K. Spangler for reviewing the literature on eosinophilia.

For many years physiologists and hematologists regarded eosinophiles as disintegrated or dead polymorphonuclear cells without any special significance. Today in studying the morphology of blood cells there is a general agreement that the polymorphonuclear eosinophilic granulocyte is a distinct cell entity and has its origin in adult life chiefly from the bone marrow, as do the polymorphonuclear neutrophiles. It has long been assumed that the specific function of the leucocyte depends upon the type of its granules. Why some granules take a neutral and others an acid or basic stain has never been satisfactorily explained.

The *neutrophilic granular leucocytes* possess ameboid movement and have phagocytic power. They contain a proteolytic ferment, are possessed of an intra- and extracellular (active in blood serum) digestive power, and are positively chemotactic for pyogenic cocci (streptococcus, staphylococcus, pneumococcus, gonococcus, etc.) whose body substances contain protein material.

The *lymphocytes*, on the other hand, which are of lymphoid origin, have a relatively sluggish ameboid movement, are not phagocytic and elaborate a lipolytic (fat splitting) ferment possessed of only intracellular digestive power (not active in the blood serum) and evidence has been adduced showing them to be positively chemotactic for tubercle bacilli and treponemes whose bodies contain fatlike substances. The fat splitting ferments of the lymphocytes have been found most active in an acid medium.

The *eosinophiles* while of myelogenic origin like the neutrophiles, possess less ameboid movement. Phagocytosis is rarely observed and eosinophiles have not been shown to produce proteolytic ferments as do the neutrophiles, or lipolytic ferments as do the lymphocytes, but apparently they have some special function related to the disintegration and removal of foreign protein from the tissues and in combating the toxins of parasites and certain bacteria. With their acid-staining granules eosinophiles would be expected to be most active in acid medium as are the lymphocytes. Eosinophiles have been shown to be responsive to the toxins of tubercle bacilli and as indicated by the findings reported in the present investigation there is clinical evidence that the eosinophile is also responsive to the toxins of the *Spirocheta pallida*.

In recent years there has been a tendency to consider most eosinophilias, even those occurring in parasitic infections, skin diseases, the exanthemas, diphtheria and certain other microbic infections as a part of the general phenomena of allergy. The clinical and immunologic significance of a relative or actual increase of eosinophile cells in the blood has remained largely conjectural and without definite interpretation.

Weber[23] states: "Eosinophilia is to be regarded as a part of the general protective allergic reaction toward the presence in the body of foreign proteins or abnormal products of albuminous catabolic metabolism." Foster[24] does not believe that the foreign protein introduced as antitoxin is responsible for eosinophilia because in a large series of cases injected routinely with serums in a hospital for contagious diseases only those of diphtheria and tuberculosis produced an eosinophilic reaction. He thinks the toxins of the diphtheria and tubercle bacilli themselves exert a positive chemotaxis on the eosinophile cells of the

hematopoietic organs. Pepper and Farley[4] state that serum disease shows no eosinophilia, which would seem to support Foster's observation.

The finding of an eosinophilia in allergic conditions, in which antibodies have not thus far been demonstrable, might possibly be regarded as immunologic evidence that allergy and anaphylaxis are not of a similar mechanism. On the other hand, eosinophilia as a common finding in anaphylactic reactions in animals[25, 26, 27, 28, 29] and also in allergic diseases in man, would seem to be an indication of a related mechanism in the two conditions.

Eosinophilia an Indication of Reactive Power of the Individual.—In giving therapeutically more than 20,000 intramuscular injections of venom (crotalin) solution, with differential blood counts made before and after injections, to more than 1,000 patients (including cases of epilepsy, asthma, hay fever, eczema, arthritis, migraine, etc.), my experience has shown that in those cases in whom an increase in the percentage of eosinophiles does not occur in from three to six weeks following weekly injections, clinical improvement of the symptoms will be doubtful. In other words, an increase of eosinophile cells following crotalin injections may be used as an indication of the reactive power of the individual and has served in my hands as a guide to dosage.

The findings of Mueller, Otfried and Broesamlen[30] following the use of tuberculin, as a nonspecific agent, correspond to my experience with crotalin. They state their investigations indicate "that the increase in eosinophile cells following an injection was the surest sign of the reactive power of the patient against the toxin."

They made counts morning, noon, and night on the day preceding, the day of the injection and the day following injection. If after three injections there continued a nonappearance of the eosinophilia, the prognosis, as to the reactive power of the patient, was not good. Hence their conclusion was "that the behavior of the eosinophilic cells can be considered as a general standard of the reactive power of the organism in its struggle against infections."

The present study of differential blood counts in patients with positive Wassermann reactions tends to indicate that a moderate eosinophilia occurs, and is discoverable if repeated counts are made, at various stages of the patient's response to the specific toxin of the parasite of syphilis, in at least 40 per cent or more of the cases. It seems reasonable to assume, therefore, that the eosinophilia occurring in syphilis may be regarded as distinct evidence of the blood's reaction to the sensitizing (toxic) substance of the *Spirocheta pallida*, and that it is indicative of the patient's development of an allergic cellular mechanism of defense.

CONCLUSIONS

1. With the hypothesis that eosinophilia is a criterion of allergy and with the finding of an eosinophilia in individuals with positive Wassermann reactions in various stages of syphilitic infection, the possible immunologic relation of allergy and syphilis would appear to justify careful study.

2. With the admittedly high incidence of unrecognized syphilitic infection and the unknown basic factor of many allergic metabolic disturbances, any clue leading to a definite diagnosis and which may aid in solving the mechanism

involved in the systemic eosinophilogenic response, would seem worthy of attention and investigation from an immunologic standpoint.

3. Finally, it seems justifiable to conclude that a moderate degree of eosinophilia, especially when associated with a lymphocytosis, occurring even periodically in an apparently nonallergic individual whose intestinal tract is free from ova and parasites, warrants the taking of Wassermann tests and the making of a persistent search for clinical evidence of syphilis.

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LABORATORY METHODS

A SIMPLE MICROMETHOD FOR THE DETERMINATION OF ALCOHOL IN BIOLOGIC MATERIAL*

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A LARGE number of investigators have proposed methods for the determination of alcohol in distillates from biologic material. The literature prior to 1927 has been summarized by Gettler and Tiber[1] and Fraenckel and Nicolai.[2] The chief developments since 1927 are the osmic acid method of Hiramatsu,[3] a modification of Stritar's alkyl iodide method by Gettler, Niederl and Benedetti-Pilcher,[4] and Bock's application of the interferometer[5] as employed by Kionka and Hirsch.[6]†

Three methods are of the microtype. They are: the bichromate-iodide method of Widmark,[7] the alkyl iodide method of Gettler, Niederl and Benedetti-Pilcher,[4] and the interferometer method. The first two are said to operate with as little as 0.1 mg. of alcohol, and in the interferometer method one scale division corresponds to 0.0005 per cent of alcohol. However, each method has certain rather serious drawbacks. In the Widmark method the starch-iodide end-point, using hundredth normal thiosulphate, is not very sharp. Gettler's ethyl iodide method is a gravimetric procedure and requires rather elaborate apparatus; also, an interferometer is expensive, and this method is invalidated by the presence of other dissolved material.

The following simple micromethod was developed in connection with a study of the metabolism of alcohol. This method operates with quantities of ethanol varying from 0.02 mg. to 0.5 mg. It is rapid and very simple, and has been in use in our laboratory and a few clinical laboratories during the past four years with entirely satisfactory results. In principle the method belongs to the bichromate group, and is a modification of certain methods referred to above.[1, 2] An excess of bichromate is employed, and, as shown by Derome and Pepin,[8] the heat developed upon adding the sulphuric acid (5 c.c. of sulphuric acid to 6 c.c. of the aqueous solution of alcohol and bichromate) is sufficient completely to oxidize the alcohol without the use of a water-bath. In titrating the excess of bichromate it was considered desirable to avoid time-consuming spot-tests or addition of excess of iodide or other reducing agent

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†Since this paper was written the iodine pentoxide method of Haggard and Greenberg (*J. Pharmacol. & Exper. Therap.* 52: 137, 1934) has appeared. This method would seem to be rather complicated in that it requires one boiling to remove iodine, and three titrations.

and back-titration with thiosulphate or permanganate. After some experimenting it was found that this could be accomplished by using a reducing solution composed of methyl orange and ferrous sulphate. In the high concentration of sulphuric acid used in the procedure, methyl orange is rapidly decolorized by even minute quantities of bichromate. In fact, methyl orange alone may be used to titrate the excess of bichromate, but the end-point is not quite so sharp as when ferrous sulphate is also present to perform about 80 per cent of the reduction. The titration is carried out in the same concentration of sulphuric acid as was used for the oxidation of the alcohol by the bichromate, thus avoiding the usual dilution and transfer of the material; and the reaction vessel, an ordinary test tube, serves both for the oxidation of the alcohol and the titration of the bichromate. The end-point is very sharp. Finally, the high concentration of sulphuric acid in the red titration fluid raises the surface tension and specific gravity so that the size of drops is greatly decreased. Thus a moderately fine buret tip will deliver about 50 drops per c.c., which somewhat increases the accuracy of the method.

Methanol may also be determined by this method, in which case the oxidation proceeds to CO_2 and H_2O, whereas, with ethanol the alcohol is oxidized to acetic acid.

APPARATUS

Reaction Tubes.—Ordinary test tubes with inside dimensions of about 17 mm. × 150 mm. are satisfactory.

Stirring Rod.—A glass rod ending in a 12 mm. ring, whose plane is at right angles with the rod.

Microburet.—A 5 c.c. microburet, which should be standardized.* To prevent the buret contents from coming in contact with the suction tube when filling from the tip, a bulb with bent tube was sealed at the top.

Device for Stirring with an Air Current During the Titration.—The stirring may be done with the stirring rod but this is rather tiresome when analyzing many samples and we prefer the following arrangement (see Fig. 1):

The tip of the microburet is drawn out to form a tube about 2.5 mm. × 35 mm. with the lower end constricted to about 1.2 mm. A rubber sleeve with a glass side arm is made from a 1¼ in. length of 5/16 in. rubber tube which is punctured about midway with a small hole. A short length of small glass tubing, flanged at one end and tapered at the other, is pushed through this hole from the inside until the flange is flush with the inner wall of the rubber tube. The rubber sleeve is now pushed over the drawn-out nozzle of the buret until the tip of the nozzle projects about ⅞ inch below the bottom of the sleeve and the upper end of the sleeve makes a tight joint with the nozzle. The glass side arm is connected with the suction pump by means of fine flexible rubber tubing running to a bent glass reducer supported by the ring stand holding the buret. A two-hole rubber stopper, which fits the reaction tube, carries in one hole a bubbler tube terminating in a bulb with small holes for breaking up the air current and in the other hole a piece of thin-walled glass tubing about 4.5 mm. wide and 18 mm. long.

*Empire Laboratory Supply Co. No. 3137 is satisfactory.

In making a titration the reaction tube is closed by means of the rubber stopper carrying the bubbler tube and is placed beneath the buret and raised so that the short glass tube is pushed over the buret tip and into the rubber sleeve until a tight joint is made and the buret tip extends about ½ inch below the lower end of the glass tube. The buret is now lowered until the reaction tube is supported on the table. A screw clamp regulates the air flow to the suction pump so that the liquid is well stirred during the titration. At the end of the titration the reaction tube and its rubber stopper are removed from the buret, and the rubber stopper and its bubbler tube transferred to the next tube to be titrated.

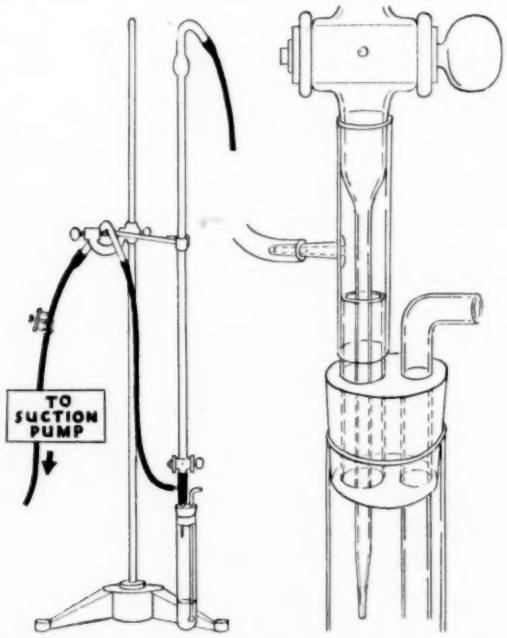

Fig. 1.—Device for stirring with an air current when many samples are to be titrated.

REAGENTS

Concentrated Sulphuric Acid.—A good grade of C. P. sulphuric acid containing very little reducing substance is employed.*

Sulphuric Acid (62%).—Pour one volume of concentrated C. P. sulphuric acid into an equal volume of water and cool.

Methyl Orange (0.1%).—Dissolve one gram of methyl orange in one liter of approximately N/40 NaOH and filter. This will keep indefinitely.

Ferrous Sulphate (20%).—Dissolve 50 grams of $FeSO_4$, $7H_2O$ in 150 c.c. of water. Add 30 c.c. of conc. H_2SO_4 and dilute to 250 c.c. In a stoppered flask this solution will undergo little oxidation for one year.

Red Reducing Fluid.—Place in a flask 35 c.c. of the 62 per cent sulphuric acid. Add to this 15 c.c. of the methyl orange solution and 1 c.c. of the fer-

*Baker & Adamson's C. P. Sulphuric Acid, No. 1180, gave very small blanks.

rous sulphate solution, shaking the flask during the additions. Mix well and cool to room temperature. This solution deteriorates slowly but will keep for three or four days, being more rapidly decomposed in warm weather. About 2.5 c.c. of this solution are required for each cubic centimeter of the standard bichromate solution.

Standard Bichromate Solution, 0.0434 N.—Dissolve 2.129 gm. of C. P. potassium bichromate in water and make up to one liter. One cubic centimeter of this solution is equivalent to 0.5 mg. of ethanol or 0.232 mg. of methanol.* The bichromate may be standardized against a weighed amount of anhydrous ethanol or in the manner described in standard works on quantitative analysis, but we have found that a good grade of C. P. analyzed bichromate (Baker and Adamson or J. T. Baker) is sufficiently pure for use as a primary standard.

REMOVAL OF ALCOHOL FROM BIOLOGIC MATERIAL

Tissues.—The material is finely hashed, mixed with an equal weight of water and acidified with a little tartaric acid. It is now steam distilled until the volume of distillate equals the weight of tissue used. One cubic centimeter of this distillate is diluted to 50 c.c., and 5 c.c. of the diluted material, representing 0.1 gm. of tissue, is used for the analysis. Where the tissue alcohol is less than 0.05 per cent the dilution of the distillate should be less than 1:50. If the tissue alcohol is above 0.5 per cent, one should use less than 5 c.c. of distillate, adding enough water to make 5 c.c.

Blood.—Since blood foams badly when steam distilled, much time is saved by first preparing a protein-free filtrate and distilling a portion of this. It was found that results by this process agree well with those obtained by steam distilling the blood. A tungstic acid protein-free filtrate of the blood is prepared by the Folin-Wu method.[9] Five cubic centimeters of this filtrate are placed in a 125 c.c. Pyrex distilling flask. To this is added 20 c.c. of water and some glass beads. The flask is connected to a small vertical condenser, the lower end of which extends well into a Folin receiving tube graduated at 25 c.c. The distilling flask is heated directly over a small flame from a microburner which is protected by a mica chimney. Distillation is continued until about 12 c.c. have come over, which requires about five minutes. The condenser tube is then rinsed out with a little water and the rinsing water collected in the receiving tube, after which the contents of the receiving tube are made up to 25 c.c. Five cubic centimeters of this distillate, representing 0.1 c.c. of blood, are used for the analysis. If the alcohol is above 0.5 per cent or below 0.05 per cent, the procedure is modified as described under "Tissues." If this distillate is to be kept some hours before analysis, it should be acidified with about six drops of concentrated H_2SO_4.

Urine.—The urine is diluted 1:10. From this point the procedure is identical with that used for a protein-free filtrate of the blood.

*Acetone causes a slight reduction of the bichromate in this method, 5 mg. of acetone consuming 0.6 c.c. of the dichromate.

ANALYSIS OF DISTILLATES

In a reaction tube place 5 c.c. of the solution of alcohol from the distillation of blood, urine or tissues, which should contain not more than 0.5 mg. of ethanol or 0.23 mg. of methanol. Add 1 c.c.* of the standard bichromate solution and then 5 c.c. of the concentrated sulphuric acid. Mix well by means of the stirring rod and allow to stand for ten minutes. Cool in water to room temperature and titrate the excess of bichromate with the red reducing fluid. The first permanent pink color should be the end-point. In the same manner run a blank on 5 c.c. of distilled water. Since sulphuric acid usually contains a trace of reducing material, the correct figure for expressing relationship of the red reducing fluid in terms of bichromate is obtained as follows: After titration add to one of the tubes a second cubic centimeter of the standard bichromate, mix the contents well and again titrate.

CALCULATION

U = titration figure for unknown.
W = titration figure for distilled water.
B = titration figure for extra c.c. of bichromate.
Q = quantity of blood, urine, or tissue represented by the aliquot analyzed.

Then, $\frac{W - U}{B} \times 0.5 \div Q =$ mg. of ethyl alcohol, per c.c. of blood or urine or gram of tissue. Where Q = 1/10 the formula becomes $\frac{W - U}{B} \times 5$. (For methanol use 0.23 instead of 0.5.)†

RESULTS WITH THE METHOD

Seventeen dilutions of absolute ethanol in water were analyzed by a student, the analyses being done in duplicate. The results are given in Table I.

TABLE I

RESULTS WITH THE METHOD (ETHANOL)*

SAMPLE	ETHANOL USED (MG. IN 5 C.C.)	ETHANOL FOUND (MG. IN 5 C.C.)	
		(A)	(B)
1	0.431	0.430	0.438
2	0.416	0.431	0.437
3	0.357	0.349	0.357
4	0.250	0.263	0.263
5	0.200	0.196	0.193
6	0.166	0.157	0.157
7	0.147	0.145	0.131
8	0.143	0.144	0.131
9	0.110	0.103	0.110
10	0.074	0.078	Lost
11	0.050	0.054	0.049
12	0.042	0.049	0.044
13	0.030	0.034	0.036
14	0.020	0.023	0.025
15	0.010	0.009	0.003
16	0.010	0.012	0.008
17	0.000	0.000	0.000

*Composition of samples unknown to analyst.

*Where the sample analyzed contains less than 0.05 mg. ethanol, the accuracy is improved by using less bichromate (0.5 c.c. or 0.2 c.c.) and reducing the amount of ferrous sulphate in the red titration fluid to 0.5 c.c. or 0.2 c.c.

†Normal blood or tissues show a small amount of reduction by this method, giving a blank of 0.02 to 0.04 c.c. of 0.0434 N $K_2 Cr_2 O_7$ for a 0.1 gm. sample. Part of this reduction is due to a volatile material in the distilled water used for diluting purposes which is concentrated in the process.

TABLE II

RESULTS WITH THE METHOD (METHANOL)*

SAMPLE	METHANOL USED (MG. IN 5 C.C.)	METHANOL FOUND (MG. IN 5 C.C.)	
		(A)	(B)
1	0.200	0.200	0.205
2	0.167	0.175	0.195
3	0.143	0.146	0.147
4	0.120	0.125	0.121
5	0.100	0.103	0.105
6	0.067	0.071	0.068
7	0.046	0.043	0.048
8	0.018	0.022	0.014
9	0.009	0.008	0.008
10	0.000	0.004	0.002

*Composition of samples unknown to analyst.

The method was also applied to aqueous solutions of methanol with the results given in Table II.

SUMMARY

1. A micromethod is described for determining alcohol in biologic material. This method operates satisfactorily with quantities of ethanol varying between 0.5 mg. and 0.02 mg.

2. The procedure is a modified bichromate method, the excess of bichromate being titrated directly with a mixture of ferrous sulphate and methyl orange.

3. The method employs very simple apparatus and consumes about as much time as a determination of blood sugar.

4. For convenience, where many analyses are required, a device is described for stirring with an air current during the titration.

5. The method will also determine methanol.

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THE STABILITY OF SUGAR IN THE CEREBROSPINAL FLUID*

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THE cerebrospinal fluid sugar has been investigated until its estimation is considered of sufficient value in the diagnosis of certain pathologic conditions to become a routine procedure in many laboratories. It has been felt generally that the estimation of this sugar should be performed at once; that otherwise the sugar would decrease in amount and the estimation become of little accurate value.

It is the purpose of this paper to test the validity of this "general feeling."

METHOD

The cerebrospinal fluid was drawn into clean, dry, sterile tubes which were stoppered and placed in an ice box at 10° C. where they remained through the period during which the estimations were made. When a quantity of fluid was desired for a sugar estimation, the tube was unstoppered and a small amount, sufficient for the estimation, was poured into another tube, thereby eliminating the necessity of sterile pipettes. The original tube was restoppered and placed in the ice box. The sugar was determined by treating the cerebrospinal fluid with 0.5 c.c. of 10 per cent sodium tungstate solution and then with 0.5 c.c. of 2/3 N sulphuric acid to precipitate the protein. After centrifugation the clear protein-free supernatant fluid (a 1:1 dilution of the original spinal fluid) was used for the determination of its sugar content by the Folin-Wu blood sugar procedure,[1] using for colorimetric comparison a Bausch and Lomb Duboscq colorimeter. The sugar estimations were run in duplicate, the average of the two estimations being used in each instance. The sugar content was expressed as milligrams of glucose per 100 c.c. of spinal fluid.

The determination on the cerebrospinal fluid sugar was done immediately after the fluid had been obtained and cooled to 10° C., and then on the following third, fifth, seventh, tenth, fourteenth, and twenty-first days. The results obtained are presented in Table I.

DISCUSSION

In this study the cerebrospinal fluids of 24 unselected individuals were used. These individuals all presented neuropsychiatric problems with and without demonstrable organic pathology. The only criterion determining the use of a fluid was its sterility. This requisite was considered as justified, and contaminated fluids were discarded.

*From the Psychiatric Clinic, Boston State Hospital.
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Throughout the period of study of these fluids, twenty-one days, the variation of the obtained values was entirely within that of the experimental method used. The largest average variation of the sugar values was 1.8 mg. and the smallest, 0.4 mg. The largest range of variation was 3.0 mg. and the smallest, 0.6 mg.

TABLE I

SPINAL FLUID SUGAR*

CASE	ON DAY PUNCTURE PERFORMED	3RD	5TH	7TH	10TH	14TH	21ST	AVERAGE	AVERAGE VARIATION	VARIATION RANGE
1	60.7	60.4	60.9	61.0	60.3	60.7	60.8	60.7	0.4	0.7
2	42.8	43.0	42.1	42.7	43.2	42.6	43.0	42.7	0.5	1.1
3	68.8	68.5	68.0	67.9	68.8	69.3	69.0	68.6	0.7	1.4
4	50.6	52.0	51.5	51.7	50.2	50.0	49.8	50.8	1.2	2.2
5	53.2	53.6	54.0	52.2	53.2	52.0	53.5	53.0	1.0	2.0
6	47.5	46.4	48.0	48.5	47.5	46.8	47.0	47.3	1.2	2.1
7	62.8	62.4	62.8	63.2	63.0	63.0	62.6	62.8	0.4	0.8
8	66.1	63.5	66.1	64.2	64.0	66.5	66.0	65.2	1.3	3.0
9	57.3	57.3	57.2	56.8	55.7	56.0	56.7	56.7	0.6	1.6
10	50.6	50.4	51.2	51.4	52.0	51.8	51.4	51.2	0.8	1.6
11	48.0	48.0	48.0	46.5	47.2	46.0	46.0	47.1	0.9	2.0
12	54.4	52.8	53.6	52.0	52.0	52.2	53.2	52.8	1.6	2.4
13	55.0	55.0	56.2	54.8	54.6	55.2	55.0	55.1	1.1	1.6
14	62.7	62.7	62.7	62.7	60.2	61.5	62.0	62.0	0.7	1.2
15	56.7	58.5	57.3	58.8	56.7	57.0	57.0	57.4	1.4	2.1
16	67.1	67.2	65.0	66.4	67.5	67.3	67.0	66.7	0.8	2.5
17	60.5	61.2	61.2	60.4	60.8	60.0	61.8	60.8	1.0	1.8
18	55.4	53.8	54.6	53.0	53.2	54.0	55.0	54.1	1.3	2.2
19	53.2	54.0	53.6	52.0	53.2	53.2	53.2	53.2	0.8	2.0
20	67.2	67.2	67.4	65.4	65.6	66.0	66.5	66.4	1.0	2.0
21	50.8	51.2	50.4	51.2	50.8	50.8	50.6	50.8	0.4	0.6
22	62.0	62.4	63.2	61.6	62.0	63.2	63.5	62.5	1.0	1.9
23	64.4	65.5	63.6	62.6	62.8	63.4	64.0	63.1	1.8	2.9
24	56.8	59.2	59.0	57.6	56.8	58.0	57.5	57.8	1.4	8.4

*Expressed in mg. per 100 c.c. cerebrospinal fluid.

Therefore, from a study of the results presented, the conclusion can be drawn that: if after the cerebrospinal fluid is obtained it is kept under sterile conditions and at a temperature of 10° C., the amount of change of the sugar therein over a period of at least twenty-one days is entirely negligible.

CONCLUSIONS

The quantity of sugar in cerebrospinal fluid kept under sterile conditions at 10° C. remains unchanged for a period of at least twenty-one days.

REFERENCE

1. Folin, O., and Wu, H.: A Simplified and Improved Method for Determination of Blood Sugar, J. Biol. Chem. **41:** 367, 1920.

ANTIGEN MIXING TUBE FOR THE KAHN TEST*

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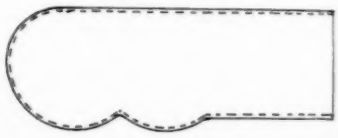
THE Kahn test has been accepted as one of the standard laboratory procedures, and has been widely adopted. One of its outstanding virtues is its adaptability in the small laboratory. In such a situation one has frequent occasion, or demand, to run at a given time only a single test, or at most from 3 to 5 tests.

The Kahn antigen, whether purchased or made in the laboratory, is a relatively expensive reagent, and the device herein described has, among other advantages, that of reducing the cost of antigen by one-half when but few serums are to be tested.

The original Kahn method requires the use of a minimum of 1 c.c. of antigen to the titrated amount of saline. These are mixed in shell vials by pouring rapidly back and forth. Due to the necessity of rapid mixing, Kahn advocates this minimum amount of antigen.



In the hands of the inexperienced, however, one frequently spills a portion, or hesitates in the process, with the resultant precipitation of the cholesterol. In the device described here the two reagents are placed in the same tube, in separate compartments, and the mixing is effected by tilting the tube and shaking. Since there is no possible loss, nor any lag in time of mixing, smaller quantities of antigen may be used.

The tube may be simply made in any laboratory having a blast burner, or even the Fisher type of burner. One takes a standard 8 by 1 inch test tube and heats the bottom of the tube, holding the tube horizontally in the flame, until well softened. Then, by cautiously blowing, a small convex blister is formed on the side of the tube just at the end. After this has cooled one heats the tube again about 1 inch higher up and blows a second pocket parallel to the first. When cooled the tube is cut off about 3 inches long.

Several attempts may be required before a satisfactory result is obtained. Each pocket should hold approximately 1 c.c. of fluid when the tube is held almost horizontal, with the pockets downward.

In use the antigen is pipetted into the bottom pocket, the saline into the upper pocket. The tube is then upended and instantly shaken, or struck against the other hand, for ten seconds. The antigen mixture is then drained into a shell vial and allowed to stand ten minutes before using.

This device has been in use in our laboratory for nine years with perfect satisfaction. We find that the use of 0.5 c.c. of antigen with the usual amount of saline (titer 1:1.1 or 1.2 c.c.) 0.55 or 0.6 c.c. gives sufficient for 7 or 8 tests.

*From the Department of Bacteriology, Medical School, The West Virginia University.
Received for publication, September 29, 1934.

This procedure thus saves one-half the antigen, which, over the course of a year, means a considerable saving in money.

A sketch of the device is appended to illustrate its simplicity.

DETERMINATION OF NITRATE IN ANIMAL TISSUES*

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IN ORDER to investigate the fate of nitrate compounds taken into the body, it was necessary to find a method suitable for analysis of animal tissues. Pucher, Vickery, and Wakeman[1] have devised a method for the determination of nitric acid in plant tissues. Their method was adapted, with certain modifications, and found to give satisfactory results when applied to animal tissues.

Preparation of Material.—The tissues were removed from the body as quickly as possible, made alkaline with 10 per cent sodium hydroxide, and dried in a drying oven, or on a steam bath. After the tissues were completely dry they were ground in a small food grinder.

Procedure.—One gram of finely ground dry tissue in a 100 c.c. beaker was mixed with sufficient 4 N sulphuric acid to bring the pH below 1. Since it was not practical for us to determine the pH of each tissue, varying quantities of acid were added to 2-gm. samples, and an analysis of each made to determine the quantity of acid necessary, and also to determine whether more than the exact amount of acid required to adjust the pH would in any way affect the results. Table I shows that at least 3 c.c. of 4 N acid are required and that amounts beyond this give the same results. It would seem that the tissue must be made distinctly acid but that an accurate adjustment of the pH is not necessary.

TABLE I

WEIGHT OF SAMPLE GM.	4N H_2SO_4 C.C.	NITRATE N MG.
2	1	0.0243
2	2	0.0775
2	3	0.54
2	4	0.48
2	5	0.59
2	6	0.58
2	10	0.585

The tissue and acid are mixed and 2.5 gm. of pure asbestos fiber are added, and the whole stirred to insure a thorough mixing. A 26 by 60 mm. Schleicher and Schüll paper extraction thimble is placed in a siphon tube (Eimer and Amend No. 30,754). A glass rod is placed in the siphon tube to hold the thimble away from the wall on one side. The transfer of material is simplified by using a 10 cm. funnel the stem of which has been cut off. The funnel fits

*From the Department of Biochemistry and Pharmacology, School of Medicine, University of Oklahoma.

Received for publication, September 13, 1934.

TABLE II

TISSUE	DOG	EXTRACTION 1 COLORIMETRIC DETERMINATION		EXTRACTION 2 COLORIMETRIC DETERMINATION		EXTRACTION 3 COLORIMETRIC DETERMINATION	
		1	2	1	2	1	2
Brain	1	0.98	1.20	0.97	0.97		
	2	1.00	1.00	1.00	1.00		
	3	0.50	0.50	0.70	0.70		
	4	0.50	0.50	0.63	0.63		
Spinal cord	1	1.44	1.40	1.43	1.30		
	2	1.26	1.26	1.33	1.33		
	3	1.00	0.97	0.93	0.93		
	4	0.82	0.82	0.90	0.90		
Trachea	1	1.83	1.83	1.80	2.00		
	2	1.47	1.47	1.63	1.63	1.38	1.38
	3	0.57	0.57	0.44	0.43		
	4	0.40	0.40	0.43	0.43		
Lung	1	0.97	0.95	0.97	0.97		
	2	1.14	1.26	1.13	1.27		
	3	0.48	0.48	0.57	0.60		
	4	0.83	0.80	0.60	0.70		
Stomach	1	1.13	1.13	1.17	1.17		
	2	0.96	0.90	0.84	0.84	0.80	0.76
	3	0.34	0.30	0.40	0.43		
	4	0.77	0.77	0.60	0.60		
Pancreas	1	1.50	1.49	1.67	1.65		
	2	1.60	1.58	1.60	1.62		
	3	0.57	0.57	0.60	0.60		
	4	0.60	0.57	0.47	0.47		
Spleen	1	1.80	2.00	2.02	2.00		
	2	1.49	1.50	1.40	1.40		
	3	0.40	0.40	0.39	0.41		
	4	0.60	0.60	0.66	0.66		
Kidney	1	1.10	1.10	1.10	1.30		
	2	1.30	1.36	1.50	1.50	1.46	1.47
	3	0.70	0.60	0.63	0.63		
	4	0.80	0.80	0.67	0.67	0.83	0.83
Intestine	1	1.00	1.05	1.00	1.00		
	2	0.76	0.76	0.60	0.60		
	3	0.56	0.56	0.70	0.70		
	4	0.30	0.30	0.40	0.30		
Muscle	1	1.77	1.67	2.00	2.00		
	2	1.30	1.33	1.36	1.27	1.47	1.47
	3	0.80	0.79	0.80	0.87		
	4	0.70	0.70	0.76	0.80		
Esophagus	1	1.23	1.20	1.27	1.27		
	2						
	3	1.33	1.33	1.07	1.10		
	4						
Skin	1	1.435	1.47	1.50	1.43		
	2	1.130	1.10	1.10	1.10		
	3	0.80	0.75	0.80	0.75		
	4	0.96	0.94				
Bladder	1	1.14	1.24	1.14			
	2	1.28	1.28	1.27			
	3	1.10	1.10				
	4	Lost					

nicely into the mouth of the thimble. The small amount of material which clings to the sides of the beaker and funnel is removed with a small piece of cotton, which is then used as a plug for the thimble. The siphon tube is attached to the spiral metal condenser of the extraction apparatus. The whole is then placed in the flask which contains 150 or 200 c.c. alcohol-free ether, and a few quartz pebbles. The apparatus is placed on an electric hot plate and the extraction conducted for eight hours. At the end of that time about 75 c.c. of hot distilled water are poured through the thimble into the flask. A few drops of phenolphthalein are added and the solution made alkaline with 10 per cent Na OH. The ether is then distilled off slowly. The residue is transferred to a 100 c.c. volumetric flask and made up to volume. Seventy-five cubic centimeters of this are transferred to a porcelain evaporating dish and evaporated to dryness. The residue is moistened with a few cubic centimeters of water, a few drops of concentrated sulphuric acid added to make the solution distinctly acid, and the whole transferred to a 25 c.c. volumetric flask. Ten cubic centimeters of 5 per cent mercuric chloride are added and the solution made up to volume by washing the evaporation dish with water, a few cubic centimeters at a time. The mercuric chloride precipitates all interfering substances and the filtrate is clear, and, except in the case of highly pigmented tissues, quite colorless. The small amount of color present does not interfere with the development or reading of the blue color produced with diphenylbenzidine. The amount of nitrate present is determined by the method of Whelan.[2]

DISCUSSION AND CONCLUSIONS

The detailed results of analysis made with this method, as a part of a study reported in another paper, are given in Table II. In all cases extractions were made in duplicate, and in some instances in triplicate. And in all cases the colorimetric determinations were made in duplicate. As the table shows, the results are close and indicate that the method determines the amount of available nitrate present.

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A MICRO-KJELDAHL TECHNIC FOR DETERMINING FIBRINOGEN*

HELEN R. GARBUTT, A.B., AND ROGER S. HUBBARD, PH.D., BUFFALO, N. Y.

IN A previous article[1] a technic was described for determining albumin and globulin in serum in which a micro-Kjeldahl oxidation and subsequent nesslerization in the presence of Rochelle salts were used. This method was quite convenient and sufficiently accurate for ordinary diagnostic purposes. It was deemed desirable to develop a similar method for determining fibrinogen, for this analysis is not technically as satisfactory as are most of those in use in diagnostic laboratories. The main objection to an application of the methods already available[2] lay in transferring the fibrin formed by the action of calcium chloride to the tube in which the micro-oxidation was to be carried out. Neither of the two procedures commonly recommended for this purpose seemed quite satisfactory. In the simultaneous destructive oxidation of filter paper and precipitate by the Folin and Wu reagent, the presence of such a large amount of carbon was undesirable, and collection of the precipitate on a glass rod and its subsequent transference to the test tube seemed to furnish opportunities for loss which should be avoided if it were possible to do so. These difficulties led us to experiment with precipitation in centrifuge tubes, and to study the most suitable method for carrying through the oxidation in the same tubes. A few minor changes in technic were found either necessary or convenient for carrying out the procedure in a satisfactory manner, and the method described below, which, in the opinion of the authors, is a very convenient one, and of a degree of accuracy well adapted to diagnostic work, was developed.

DESCRIPTION OF METHOD

Apparatus.—(1) Conical 50 c.c. centrifuge tubes of Pyrex glass graduated at 10 c.c., (2) microburner, and (3) ring stand with adjustable clamp to hold centrifuge tubes.

Reagents.—(1) Eighty-five hundredths per cent sodium chloride, (2) 2.5 per cent calcium chloride, (3) 0.05 per cent calcium chloride, (4) the dilute 1:1 sulphuric acid and phosphoric acid digestion mixture of Folin and Wu,[3] (5) 10 per cent Rochelle salts, (6) Nessler's solution prepared according to the directions of Folin and Wu,[3] (7) a strong ammonium sulphate standard containing 0.1414 gm. of pure salt in 100 c.c. of water, and (8) a weak ammonium standard prepared by diluting 10 c.c. of the one just described to 100 c.c.

Method.—Place 0.5 c.c. of plasma in a Pyrex centrifuge tube graduated at 10 c.c. Add 24 c.c. of 0.85 per cent sodium chloride and 0.5 c.c. of 2.5

*From the Buffalo General Hospital.
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per cent calcium chloride solution. (These are the reagents recommended by Cullen and Van Slyke.[4]) Let stand until the clot has formed, which usually takes about an hour. Centrifuge at high speed until the clot has packed into the bottom of the tube. Decant the supernatant liquid. Wash twice with 5 c.c. portions of 0.05 per cent calcium chloride solution. Add 2 c.c. of the dilute oxidizing mixture of Folin and Wu containing sulphuric and phosphoric acids. Heat the tube over a low flame, shaking constantly to prevent bumping, until the precipitate has dissolved in the digestion mixture. Clamp the tube in a slanting position and heat carefully with a microburner until all the water has been driven off and white fumes appear. Then place the tube in a vertical position, cover with a watch glass, and continue heating until oxidation is complete and the contents of the tube have become colorless. Let cool until the fumes have almost wholly subsided, then tilt the tube and dilute with distilled water from a medicine dropper, pouring the water carefully down the side of the tube, adding only a drop at a time at first and more rapidly later. Let cool. If a crystalline precipitate has formed at this point, it may be broken up with a glass rod and the rod rinsed into the tube. Make the volume to 10 c.c., and centrifuge if necessary. Transfer 5 c.c. into a second tube graduated at 10 c.c., add 1 c.c. of 10 per cent Rochelle salts, make up to 10 c.c., and nesslerize with 15 c.c. of the reagent described by Folin and Wu. Simultaneously nesslerize standard solutions containing known amounts of ammonium sulphate, 1 c.c. of the dilute oxidizing reagent of Folin and Wu, and 1 c.c. of 10 per cent solution of Rochelle salts in a total volume of 10 c.c. Convenient standards contain 0.15, 0.09, and 0.06 mg. of nitrogen. These amounts are contained in 5, 3, and 2 c.c. of the dilute standard described above. Compare the color of the unknown solution with that of the standard nearest to it in tint in a colorimeter, and calculate the percentage of the fibrinogen present by means of the following formula:

$$\text{Per cent fibrinogen} = \frac{\text{reading of standard}}{\text{reading of unknown}} \times \text{value of standard} \times \frac{400}{1000} \times 6.25$$

STANDARDIZATION OF METHOD

Various experiments were carried out in attempting to standardize the method. The accuracy of the oxidation technic was established by precipitating proteins from blood serum and from alkaline solutions of various kinds (egg white, pollens, etc.) and comparing the results obtained by the direct procedure described with those given by the method of Hubbard and Sly.[1] The agreement in these experiments was very satisfactory.

The probable efficacy of washing with calcium chloride as a method of separating the precipitated protein from interfering nitrogenous compounds was also investigated. It was found that one treatment with calcium chloride was probably sufficient, for no nitrogen was found in the supernatant fluid from subsequent washings, and the nitrogen content of the precipitate after one, two, and three washings was identical. Nevertheless, to guard against possible error, it has seemed best to recommend the double treatment described.

As a convenient method of testing the accuracy of the method, we made a comparison between fibrin determinations made as described and studies based upon the difference between the total nitrogen content of plasma and of plasma from which fibrinogen had been removed.[5] The results of these studies were quite unsatisfactory. There was a fair degree of parallelism between the results of the two methods, but individual values often differed markedly from each other. In some experiments the determination described in this paper gave the higher value, and in others the reverse was true. The explanation of the cause of these discrepancies was obvious, for the actual value of the difference upon which the comparison was based was often very little greater than the algebraic sum of the errors inherent in the two micro-Kjeldahl analyses upon the treated and untreated plasma.

It was, therefore, necessary to adopt some other plan for testing the probable accuracy of the proposed method, and a comparison of results on varying amounts of plasma was accordingly made. In these studies no variations were introduced into the procedure, except that the total volume of salt plus plasma was kept constant and that the number of standards prepared was increased to allow for the large range of nitrogen values encountered. The results of these experiments are given in Table I.

TABLE I

VALUES OBTAINED IN DUPLICATE DETERMINATIONS ON VARYING AMOUNTS OF PLASMA

(Values expressed as gm. fibrinogen per 100 c.c. plasma)

SERIAL NO.	1 C.C. TAKEN	0.5 C.C. TAKEN			0.25 C.C. TAKEN
Plasma 1	--	0.53%	0.53%		--
2	--	0.69%	0.53%		--
3	--	0.43%	0.43%		--
4	--	0.57%	0.56%		--
5	--	0.36%	0.35%		--
6	--	0.42%	0.43%		--
7	--	0.89%	0.87%	0.91%	--
8	0.43%	0.42%			0.50%
9	0.20%	0.21%			--
10	0.56%	0.51%			0.51%
11	0.27%	0.30%			0.28%
12	0.42%	0.39%			0.41%
13	0.44%	0.44%			0.41%
14	--	0.56%			0.56%
15	0.36%	--			0.37%
16	0.43%	--			0.42%
17	0.23%	0.22%			0.22%
18	0.65%	0.57%			0.59%
19	0.60%	0.50%			0.55%
20	0.53%	0.46%			--
21	0.35%	0.35%			--
22	0.23%	0.24%			0.23%
23	0.29%	0.29%			0.27%
24	0.41%	0.41%			--
25	0.23%	0.23%			--
26	0.35%	0.35%			0.31%
27	0.37%	0.37%			0.35%
28	0.52%	0.56%			--
29	0.14%	0.14%			0.14%
30	0.11%	0.11%			--
31	0.46%	0.48%			0.48%
32	0.33%	0.33%			--

TABLE II

PER CENT DIFFERENCE BETWEEN PAIRS OF VALUES

PAIRS COMPARED	RANGE OF PER CENT DIFFERENCE				
	0 TO 2% NO.	3% TO 5% NO.	6% TO 10% NO.	10% TO 15% NO.	OVER 15% NO.
0.5 with 0.5 c.c.	6	2	1	–	–
0.5 with 1.0 c.c.	11	4	4	2	1
0.5 with 0.25 c.c.	5	4	4	2	–
0.25 with 1.0 c.c.	3	5	5	2	–
Total	25	15	14	6	1

It is evident that in general the agreement shown by these determinations was quite satisfactory. Table II has been prepared to show just how close this agreement was. In this table is given the number of pairs agreeing closely together, those showing differences between 2 and 5 per cent, etc. The table shows that even under these rather extreme conditions the majority of the pairs of determinations showed differences of less than 5 per cent. The average of all the values was 4.3 per cent. Such a degree of accuracy is sufficiently great for a method designed for use in a diagnostic laboratory.

CONCLUSION

A method of determining fibrinogen, which renders transference of the precipitated material unnecessary, is described. The method gave results having a degree of accuracy sufficiently great to justify its use in routine work.

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A NEW CLINICAL MODEL OF THE HADEN-HAUSSER HEMOGLOBINOMETER*

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IN 1930[1] I described a new hemoglobinometer which was developed in collaboration with the manufacturers, C. A. Hausser and Son. I have shown that hemoglobin estimations with this instrument check closely with determinations made by the oxygen capacity and iron content methods.[2] Our favorable experience with the Haden-Hausser hemoglobinometer has been verified in many other laboratories. The one objection to the instrument has been that it is essentially a laboratory instrument by reason of its size and original cost.

To overcome the objections of size and expense, a clinical model was later described.[2] This instrument has not proved as satisfactory as was hoped since

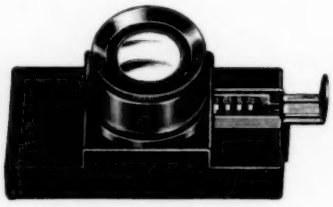

Fig. 1.

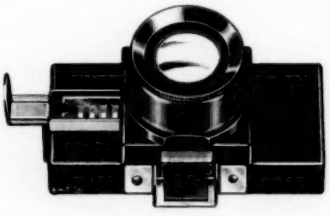

Fig. 2.

Fig. 1.—Front view of instrument, showing comparator slide in position.
Fig. 2.—Rear view of instrument, showing light filter in position.

it is somewhat cumbersome to use, is fragile, and is not as sensitive as the laboratory model. Because of these difficulties, the model described herewith has been developed. It is essentially the laboratory model in a smaller size and without the light box. It is easily transported, it is inexpensive, and the readings are as accurate as those of the larger model.

The instrument is shown in Figs. 1 and 2, and in cross-section in Fig. 3. The molded Bakelite case holds the reading microscope, the movable comparator slide (Fig. 4) and the mirror for reflection of light from an outside source through the light filter. Filters for both daylight and artificial light are supplied. The comparator slide, except for the smaller dimensions, is exactly the same as the one used in the laboratory model and it is sealed in the metal carrier. The slide has a hemoglobin gram scale with a total range

*From the Cleveland Clinic.
This instrument is made by C. A. Hausser and Son and is sold by Arthur H. Thomas Company, West Washington Square, Philadelphia.

from 7.5 to 18 gm. in the following intervals: 7.5, 8, 8.5, 9, 9.5, 10, 11, 12, 13, 14, 15, 16, 17, and 18 gm. This scale was arrived at by determining the oxygen combining capacity of many samples of blood throughout the range of the scale by use of the Van Slyke manometric blood gas method converted into hemoglobin content by the use of the Hüfner conversion factor of 1.34 ml. of oxygen per gram of hemoglobin. The glass used in the standard is the same as that shown by Razek[3] to have an absorption curve almost identical with acid hematin (Fig. 4). The comparator slide consists of a glass color standard of the same thickness throughout, which is in immediate juxtaposition to a wedge-shaped channel *E* for the dilution complement. This comparator is moved through the field of the reading microscope as the comparison is made. A series of illuminated rectangles, separated by alternate dark spaces,

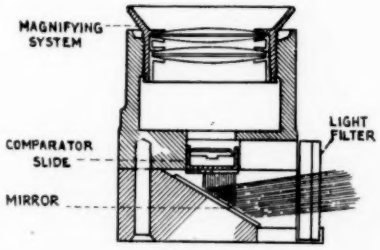

Fig. 3.

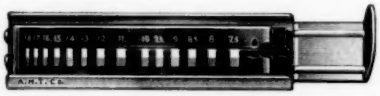

Fig. 4.

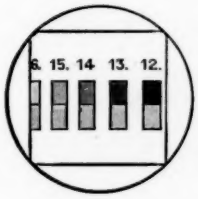

Fig. 5.

Fig. 3.—Cross-section view of instrument.
Fig. 4.—Comparator slide with cover glass in position.
Fig. 5.—Field of view in the new clinical model reading 14 grams.

then appears in the field of the microscope. The lower half of each rectangle constitutes the color standard complement, while the upper half of each rectangle constitutes the dilution complement when the wedge-shaped chamber is filled with diluted blood. The depths of the dilution complements vary because of the wedge-shaped channel.

In making hemoglobin determinations, the blood is diluted 1:20—or 1:10 if the hemoglobin content is probably below 7.5 gm., i.e., approximately 50 per cent—in a Thoma white cell pipette with N/10 hydrochloric acid. This low dilution ratio results in greater accuracy than is possible with the higher dilutions required in some instruments. After conversion of the hemoglobin into acid hematin, the diluted blood is run into the wedge-shaped dilution channel of the comparator at the end of the cover glass. The channel fills by capillarity.

An approximate determination of the hemoglobin is made by moving the comparator holder to the left until the upper illuminated half, the dilution complement, of the rectangle observed in the center of the field closely matches the glass color standard complement below. The rectangle in which the complements most nearly match is best determined by making such an approximate selection and then contrasting the upper complement with the adjacent color to the left and again with the adjacent color to the right. With the correct rectangle in the center of the field, the dilution complement to the left will be of lighter tint and the dilution complement to the right of a darker tint, and the dilution and color standard complements of this central rectangle will closely match.

The field of view in the new clinical model set for a reading of 15 gm. is shown in Fig. 5.

I have made comparative readings with this new instrument and with the laboratory model with results as shown in Table I.

TABLE I

SPECIMEN NO.	NEW CLINICAL MODEL GM.	LABORATORY MODEL GM.
1	12	12
2	14	14
3	10	10
4	16	16
5	13	13
6	11	11

In calculating the hemoglobin in percentage, any one of the hemoglobin standards can be used. A reading of 15 gm. would be transposed into percentage as follows:

By Haden's standard[4] $\frac{15.0 \times 100}{15.4} = 97\%$

By Wintrobe's standard[5] $\frac{15.0 \times 100}{14.5} = 103\%$

By Osgood's standard[6] (for men) $\frac{15.0 \times 100}{14.7} = 102\%$

By American Society of Clinical Pathologist's standard $\frac{15.0 \times 100}{17.3} = 87\%$

By standard determined locally in example given below $\frac{15.0 \times 100}{15.4} = 97\%$

Since the number of grams of hemoglobin per 100 c.c. calculated to a count of 5 million cells has been found to vary in different laboratories and localities, it is desirable that each laboratory determine locally the standard to be used. The determination is simply, and fairly accurately done by making careful erythrocyte counts and hemoglobin estimations in grams on ten healthy, normal adults, although it is preferable to have a larger number. From data obtained, the number of grams of hemoglobin per 100 c.c. per 5 million red cells is calculated as indicated in Table II, and this figure is taken as 100 per cent of normal.

TABLE II

PATIENT	RED CELL COUNT PER C.MM. MILLIONS	HEMOGLOBIN PER 100 C.C. GRAMS
1	5.02	15.5
2	4.00	12.6
3	4.65	14.9
4	5.50	16.8
5	4.78	15.0
6	4.57	14.2
7	4.25	13.5
8	5.31	16.5
9	5.80	16.9
10	4.92	15.1
Mean of 10	4.88	15.1

The hemoglobin (X) per 5 million cells is

$$5.00 \times \frac{15.1}{4.88} \text{ or } 15.4 \text{ gm.}$$

This figure is taken as 100 per cent for the laboratory in which the data are obtained and when so used should give a color index of 1.00 ± 0.10 in normal adults. The standards suggested by Haden, Wintrobe, and Osgood have been determined by this method but a much larger number of determinations have been made to diminish the error of the mean.

SUMMARY

A simplified model of the Haden-Hausser hemoglobinometer is described which is inexpensive, accurate, and simple to use.

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TENTH NORMAL HYDROCHLORIC ACID AS A DILUTING FLUID FOR COMBINED LEUCOCYTE AND HEMOGLOBIN DETERMINATIONS*

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FOR use in the Haden-Hausser hemoglobinometer a 1-20 dilution of blood in tenth normal hydrochloric acid is made in a white cell diluting pipette. An economy of time and apparatus would result if the white cells could be counted in the same suspension.

Using a single sample of well-mixed oxalated blood, ten white cell counts were made in duplicate with tenth normal hydrochloric acid and with 0.5 per cent acetic acid as diluents. When subjected to standard statistical analysis, no significant difference was found between the means of the two series. Other samples were counted in duplicate and in no instance was there a greater difference than in the first series. Variation in the distribution of cells within the counting chamber was found sufficient to account for the major portion of the difference between the two series.

Ten suspensions in hydrochloric acid recounted after standing at least two hours showed good keeping qualities of the diluent, the second series being 8.7 per cent higher than the first.

Fifty duplicate counts on clinical specimens with both diluents showed an average deviation of 6.09 per cent.

The method has been used routinely for six months with perfect satisfaction.

The acid hematin suspension produces a light brown background and the cells are a little smaller in hydrochloric acid, but this is not sufficient to interfere with the accuracy of the counts.

A Reider pipette is found to be preferable to the Thoma because of its larger volume and greater potential accuracy. An effort was made to develop a special pipette for this purpose. However, objections to a pipette requiring a large volume of blood forced us to abandon this idea. We are indebted to Mr. S. Trenner, of Arthur H. Thomas and Company, Philadelphia, for aid with this pipette.

CONCLUSION

Tenth normal hydrochloric acid is a suitable diluent for making white cell counts. The same preparation may be used for this purpose and for hemoglobin determinations.

We were pleased to learn that Dr. Frederick Boerner, of Philadelphia, was developing this idea at the same time that we were, and that his conclusions are the same as ours. We have just learned also that Walker† used

*From the Department of Pathology, Fitkin Memorial Hospital, Asbury Park, N. J. and the Monmouth Memorial Hospital, Long Branch, N. J.

Received for publication, September 15, 1934.

†Walker, M. A.: Tenth-Normal Hydrochloric Acid as Diluent for Counting Leukocytes After Infusion of Solution of Acacia, Am. J. Clin. Path. 2: 347, 1932.

tenth normal hydrochloric acid for white cell counts after acacia injections, but did not advocate its routine use.

We wish to thank Dr. H. M. Lufkin, Instructor in Mathematics, University of Pennsylvania, for the statistical analysis.

THE PERCENTAGE OF HEMOGLOBIN COMPARED TO THE VOLUME OF ERYTHROCYTES*

The Importance of this Relation in Correcting the van Allen Determination of the Volume of Platelets

Kaare K. Nygaard, M.D., and Doris L. Duxbury, B.A., Rochester, Minn.

THOMSEN, in 1919, published a report on a direct method for performing platelet counts. This method was based on the observation that platelets are evenly suspended in a plasma oxalate solution for a certain length of time (up to four hours), whereas the erythrocytes and leucocytes during this time will settle, thus leaving a clear oxalate plasma solution from which a drop may be transferred to a regular chamber for counting. Because of the dilution of the blood with oxalate solution, and because of the volume of the settled erythrocytes and leucocytes which will influence the dilution, it is necessary to correct the platelet count, and this necessitates determination of the hematocrit value in each case.

Gram, in 1921, simplified the correction. In 611 cases of disease of all kinds he found that there was constant and striking parallelism between the percentage of hemoglobin and the volume of cells. By determining the percentage of hemoglobin he could, therefore, obtain a good idea of the corresponding volume of cells, and the correction of the platelet count consequently could be based on determination of the percentage of hemoglobin.

Van Allen in 1925, published a report on his method of determining the volume of platelets; his method was based on the same principle as that applied in Thomsen's method for counting platelets. Consequently, it is necessary to apply a correction to the volume of platelets, as well as to the count in Thomsen's method, since the correction value is different and depends on the dilutions of blood oxalate solution. We have computed the correction for the platelet volume for any value as determined by hematocrit and have found it to vary from 2 per cent in a case in which the hematocrit determination was 10 per cent, to 15 per cent in a case in which the hematocrit gave a value of 75 per cent.

Again, taking up Gram's idea of substituting the hematocrit determination for the corresponding hemoglobin value, we have sought to simplify this

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*From the Mayo Clinic.

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correction. Gram, however, has not given any specific data regarding the relation of hemoglobin to volume of cells. We were not able to find such correlation values in the literature. This work, therefore, was undertaken in the hope that it would be of some practical importance in correcting platelet counts, and probably also similar questions that should arise in the future. Dr. H. L. Dunn, gave authoritative advice throughout this study.

Rowntree and Brown with the assistance of Roth, made simultaneous observations of different phases of the blood in a large series of cases. With the authors' permission we have gathered from their monograph simultaneous determinations of hemoglobin and of hematocrit values. The value for hemoglobin in grams per 100 c.c. of blood was determined according to a method which in reality was a combination of the method of Haden and that of Osgood, using Haden's standard and Osgood's technic.

The principle is the addition of dilute hydrochloric acid to whole blood, whereby oxyhemoglobin is converted to acid hematin. Comparison is undertaken with a standard of acid hematin in a colorimeter.

The volume of erythrocytes was determined by adding 5 c.c. of blood to 1 c.c. of a 1.6 per cent solution of sodium oxalate in an accurately graduated centrifuge tube and by centrifuging for thirty minutes at 3,000 revolutions per minute. "The volume of the corpuscles is expressed as a percentage of the volume of the sample of blood drawn."

TABLE I

OBSERVATIONS MADE BY ROWNTREE AND BROWN WITH THE ASSISTANCE OF ROTH

	CASES	OBSERVATIONS
Normal men of varying body build	48	48
Normal women of varying body build	25	25
Normal subjects, whose blood volume and blood plasma fluctuated	22	44
Obesity	26	26
Chronic secondary anemia	15	15
Pernicious anemia	9	9
Polycythemia vera	49	49
Myelogenous leucemia	10	11
Lymphatic leucemia	4	4
Splenomegaly without anemia	6	6
Cirrhosis of the liver with splenomegaly	9	9
Hemolytic jaundice	11	11
Primary splenomegaly with anemia	18	18
Glomerulonephritis with edema	12	12
Subacute glomerulonephritis with developing anemia and incomplete diuresis	1	9
Nephrosis in the stage of edema	9	9
Cardiac edema	11	11
Diabetes with edema	3	6
Essential hypertension	10	10
Raynaud's disease	8	11
Thromboangiitis obliterans	5	5
Arteriosclerotic disease with occlusion	3	3
Arteriovenous fistula	7	7
Myxedema	8	8
Hyperthyroidism	4	4
Addison's disease	12	18
Diabetes insipidus	4	5
Diabetes mellitus	7	7
Chronic bronchitis, emphysema, and asthma	12	12
Total		412

The number of observations made by Rowntree and Brown (and Roth) and the type of cases they employed are given in Table I.

The distribution of the reading of grams of hemoglobin per 100 c.c. of blood, and the hematocrit value, are included in Table II.

The mean value for hemoglobin was 15.131 gm. The standard deviation was ± 4.182 gm. The mean hematocrit value was 41.966 per cent, and the standard deviation, ± 10.455 per cent. If x represents grams of hemoglobin per 100 c.c. of blood, and if y represents the hematocrit value in per cent, the coefficient of correlation, r, between x and y was found to be 0.885.

This is a very high correlation coefficient, indicating close correlation between grams of hemoglobin per 100 c.c. blood and the hematocrit value, thus substantiating Gram's statement. With this correlation definitely worked out, it was desired statistically to compute from any given value for grams of hemoglobin per 100 c.c. of blood, the correlated hematocrit value. The linear equation for this can be easily obtained from the constants already given and was found to be $y = 2.2136\,x + 8.47$.

From any known value for grams of hemoglobin per 100 c.c. of blood x the correlated hematocrit value y can, therefore, be computed, according to the formula. Table II gives the result of this computation.

It will be easily seen that the correction values of Van Allen's platelet volume, computed on the basis of hematocrit values, can now readily be based on the corresponding number of grams of hemoglobin, thus obviating the hematocrit determination.

Aside from this practical point, the computation has revealed a theoretic problem of some importance. The high correlation coefficient found between grams of hemoglobin per 100 c.c. of blood and the hematocrit value, indicates that in the material concerned there is a constant amount of hemoglobin per volume unit of erythrocytes. This is another way of expressing what is termed the hemoglobin saturation of erythrocytes. Naegeli has pointed out that this hemoglobin saturation is maintained with surprising consistency, and is found to be a maximal one in normal cases. In many cases of severe anemia (chlorosis) this saturation is found to be decreased. In pernicious anemia there is supposed to be a maximal saturation. The material we have used does not include any cases of chlorosis; but does include a considerable group of anemias of different kinds. That the correlation coefficient is found as high as 0.86– seems to indicate that the hemoglobin saturation per volume unit of erythrocytes in this material is maintained with rather remarkable persistence. We find, however, that for further information, the method of study here applied ought to be applied to larger and separate groups of different kinds of diseases.

SUMMARY

Based on the simultaneous observations of grams of hemoglobin per 100 c.c. of blood and the hematocrit value in 412 determinations, made of normal persons and of patients with various diseases, a very close correlation has been found between grams of hemoglobin and the hematocrit value. The coefficient of correlation between these two ($r = 0.885$) has been computed.

TABLE II

RESULTS OF COMPUTATION

HEMOGLOBIN, GM.	HEMATOCRIT, PER CENT	REDUCTION PLATELET VOLUME, PER CENT	HEMOGLOBIN, GM.	HEMATOCRIT, PER CENT	REDUCTION PLATELET VOLUME, PER CENT	HEMOGLOBIN, GM.	HEMATOCRIT, PER CENT	REDUCTION PLATELET VOLUME, PER CENT
		3.0	9.0	28.4		15.0	41.7	
			9.1	28.6		15.1	41.9	
			9.2	28.8		15.2	42.1	8.5
			9.3	29.1		15.3	42.3	
			9.4	29.3		15.4	42.6	
			9.5	29.5		15.5	42.8	
			9.6	29.7		15.6	43.0	
			9.7	29.9	6.0	15.7	43.2	
			9.8	30.2		15.8	43.4	
			9.9	30.4		15.9	43.7	
4.0	17.3		10.0	30.6		16.0	43.9	
4.1	17.5		10.1	30.8		16.1	44.1	
4.2	17.8		10.2	31.1		16.2	44.3	
4.3	18.0	3.6	10.3	31.3		16.3	44.5	
4.4	18.2		10.4	31.5		16.4	44.8	
4.5	18.4		10.5	31.7		16.5	45.0	9.0
4.6	18.7		10.6	31.9	6.5	16.6	45.2	
4.7	18.9		10.7	32.2		16.7	45.4	
4.8	19.1		10.8	32.4		16.8	45.7	
4.9	19.3		10.9	32.6		16.9	45.9	
5.0	19.5		11.0	32.8		17.0	46.1	9.2
5.1	19.8		11.1	33.0		17.1	46.3	
5.2	20.0	4.0	11.2	33.3		17.2	46.5	
5.3	20.2		11.3	33.5		17.3	46.8	
5.4	20.4		11.4	33.7		17.4	47.0	
5.5	20.6		11.5	33.9		17.5	47.2	
5.6	20.9		11.6	34.1		17.6	47.4	
5.7	21.1		11.7	34.4		17.7	47.7	
5.8	21.3		11.8	34.6		17.8	47.9	
5.9	21.5		11.9	34.8		17.9	48.1	9.6
6.0	21.7		12.0	35.0	7.0	18.0	48.3	
6.1	22.0	4.5	12.1	35.2		18.1	48.5	
6.2	22.2		12.2	35.5		18.2	48.8	
6.3	22.4		12.3	35.7		18.3	49.0	
6.4	22.6		12.4	35.9		18.4	49.2	
6.5	22.9		12.5	36.1	7.2	18.5	49.4	
6.6	23.1		12.6	36.4		18.6	49.6	
6.7	23.3		12.7	36.6		18.7	49.9	
6.8	23.5		12.8	36.8		18.8	50.1	10.0
6.9	23.7		12.9	37.0		18.9	50.3	
7.0	24.0		13.0	37.3		19.0	50.5	
7.1	24.2		13.1	37.5		19.1	50.7	
7.2	24.4		13.2	37.7		19.2	51.0	
7.3	24.6		13.3	37.9		19.3	51.2	
7.4	24.9		13.4	38.1	7.6	19.4	51.4	
7.5	25.1	5.0	13.5	38.3		19.5	51.6	
7.6	25.3		13.6	38.6		19.6	51.9	
7.7	25.5		13.7	38.8		19.7	52.1	
7.8	25.7		13.8	39.0		19.8	52.3	
7.9	26.0	5.2	13.9	39.2		19.9	52.5	
8.0	26.2		14.0	39.5		20.0	52.7	
8.1	26.4		14.1	39.7		20.1	52.9	
8.2	26.6		14.2	39.9		20.2	53.2	
8.3	26.8		14.3	40.1	8.0	20.3	53.4	
8.4	27.1		14.4	40.3		20.4	53.6	
8.5	27.3		14.5	40.6		20.5	53.9	
8.6	27.5		14.6	40.8		20.6	54.1	
8.7	27.7		14.7	41.0		20.7	54.3	
8.8	27.9	5.6	14.8	41.2		20.8	54.5	
8.9	28.2		14.9	41.4		20.9	54.7	

TABLE II—CONT'D

HEMOGLOBIN, GM.	HEMATOCRIT, PER CENT	REDUCTION PLATELET VOLUME, PER CENT	HEMOGLOBIN, GM.	HEMATOCRIT, PER CENT	REDUCTION PLATELET VOLUME, PER CENT	HEMOGLOBIN, GM.	HEMATOCRIT, PER CENT	REDUCTION PLATELET VOLUME, PER CENT
21.0	55.0	11.0	24.2	62.0		27.4	69.1	
21.1	55.2		24.3	62.3		27.5	69.3	
21.2	55.4		24.4	62.5		27.6	69.6	
21.3	55.6		24.5	62.7		27.7	69.8	
21.4	55.8		24.6	62.9		27.8	70.0	14.0
21.5	56.1		24.7	63.1		27.9	70.2	
21.6	56.3		24.8	63.4		28.0	70.5	
21.7	56.5		24.9	63.6		28.1	70.7	
21.8	56.7		25.0	63.8		28.2	70.9	
21.9	56.9		25.1	64.0		28.3	71.1	
22.0	57.2		25.2	64.3		28.4	71.3	
22.1	57.4		25.3	64.5		28.5	71.6	
22.2	57.6		25.4	64.7		28.6	71.8	
22.3	57.8		25.5	64.9		28.7	72.0	
22.4	58.1		25.6	65.1	13.0	28.8	72.2	
22.5	58.3		25.7	65.4		28.9	72.4	
22.6	58.5		25.8	65.6		29.0	72.7	
22.7	58.7		25.9	65.8		29.1	72.9	
22.8	58.9		26.0	66.0		29.2	73.1	
22.9	59.2		26.1	66.2		29.3	73.3	
23.0	59.4		26.2	66.5		29.4	73.5	
23.1	59.6		26.3	66.7		29.5	73.8	
23.2	59.8		26.4	66.9		29.6	74.0	
23.3	60.1	12.0	26.5	67.1		29.7	74.2	
23.4	60.3		26.6	67.3		29.8	74.4	
23.5	60.5		26.7	67.6		29.9	74.7	
23.6	60.7		26.8	67.8		30.0	74.9	15.0
23.7	60.9		26.9	68.0				
23.8	61.1		27.0	68.2				
23.9	61.4		27.1	68.5				
24.0	61.6		27.2	68.7				
24.1	61.8		27.3	68.9				

TABLE III

HEMOGLOBIN AND HEMATOCRIT READINGS (ROWNTREE AND BROWN WITH ASSISTANCE OF ROTH)

HEMATOCRIT, PER CENT	HEMOGLOBIN PER 100 C.C. OF BLOOD, GM.*														
	0 to 1.9	2 to 3.9	4 to 5.9	6 to 7.9	8 to 9.9	10 to 11.9	12 to 13.9	14 to 15.9	16 to 17.9	18 to 19.9	20 to 21.9	22 to 23.9	24 to 25.9	26 to 27.9	28 to 29.9
10 to 14			2	1			1								
15 to 19				1	2										
20 to 24			1	6	6	4	1								
25 to 29				4	10	11	4								
30 to 34				1	4	10	17	15	1						
35 to 39					2	10	27	38	11	2					
40 to 44						2	13	62	40	14	1				
45 to 49				1			3	11	15	6	4				
50 to 54								1	2	2			1		
55 to 59								1		3	2	2	1		
60 to 64								1			6	2	2	1	1
65 to 69										1	2	4	1	4	1
70 to 74												2		1	2
75 to 79													1	1	

*Quantities in body of table represent cases.

Applying Karl Pearson's product-moment method of computing correlations, it has been possible to compute from any known value of grams of hemoglobin per 100 c.c. of blood, the correlated hematocrit value. The correlation is published in the form of a table. The practical applicability of such a table is clear when information is sought about two values and when determination of only one of the correlated values concerned is performed. An example is given in applying the table to the correction of the van Allen determination of platelet volume. The hemoglobin saturation of the volume unit of the erythrocytes is briefly discussed and in this series of 412 determinations is assumed to be remarkably constant.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

DEXTROSE, Effects of Intravenous Administrations of Hypertonic Solutions of, Masserman, J. H. J. A. M. A. **192**: 2084, 1934.

The effects of the intravenous injection of solutions of dextrose in various amounts and concentrations were studied in 85 normal patients. The administration of 50 gm. or less in 20 per cent solution produced no untoward clinical sequelae other than diuresis; however, the intravenous injection of 100 gm. or more in 35 to 50 per cent solution caused headaches and other adverse symptoms in 72 per cent of cases, whereas 58 per cent of the patients receiving 185 gm. or more suffered transient pyrexia. The intravenous administration of isotonic solutions caused a transient increase in cerebrospinal fluid pressure; with hypertonic solutions in effective concentration (100 to 200 gm. in 20 to 35 per cent solution) this initial rise was followed by a secondary fall in cerebrospinal fluid pressure, which in turn was superseded within an average of three hours by a tertiary increase to levels from 8 to 148 mm. of water above normal. The latter phenomenon is of clinical significance in relation to the late adverse effects sometimes observed in cases of intracranial hypertension treated by the intravenous injection of strongly hypertonic solutions.

BILIRUBIN, Ernst-Foster Method of Measuring in the Blood, Gajdos, A. Rev. Med.-Chir. des Malad. du Foie **9**: 45, 1934.

Gajdos believes the Ernst-Foster method superior to Van den Bergh's in the determination of bilirubinemia, especially when the concentration in the blood is low.

The method follows:

1. To 1 c.c. of serum or plasma add 2 c.c. of acetone.
2. Mix well, filter, and centrifuge the filtrate.
3. Compare the supernatant fluid either in a colorimeter with a 1:6,000 dilution of potassium bichromate (representing 0.329 mg. of bilirubin per 100 c.c.) or against a series of standards prepared by graded dilutions.
4. Multiply the reading by 3.

If the bilirubin concentration is expected to be low only 1.5 c.c. of acetone is added to the serum, the reading then being multiplied by 2.5.

CYCLOMASTOPATHY: A Physio-Pathological Conception of Some Benign Breast Tumors, With an Analysis of Four Hundred Cases, Oliver, R. R., and Major, R. C. Am. J. Cancer **31**: 1, 1934.

In this review the development of the breast has been considered, together with the histologic changes accompanying the initiation, course, and completion of the various cycles to which the organ is subject. A number of opportunities for abnormalities in behavior of the involved tissues have been demonstrated.

The dependence of the breast gland upon a complicated hormonal stimulation has been proved by work, the literature of which has been reviewed.

The historical aspects of benign tumors of the breast have been considered together with the development of an involved terminology.

The term "cyclomastopathy" is offered as a designation for the entire group of breast affections which present excessive connective tissue or epithelial proliferation, or both, in response to growth stimuli or as a manifestation of abnormal involution following normal response.

The term "eccyclomastoma" is offered as a designation for localized areas of cyclomastopathy which give rise to palpable masses or to symptoms. The group of cases included in this study are examples of eccyclomastoma.

Four hundred cases of benign breast lesions have been analyzed.

While not admitting of definite proof, it is the author's impression that eccyclomastoma occurs more frequently in white than in colored women.

The peak of age incidence for all cases is reached between the twentieth and twenty-fifth years. The peak of incidence for white women is reached in the same age period; for colored women, in the period from fifteen to twenty years. The peak of incidence for both "fibro-adenoma" and "intracanalicular myxoma" groups is reached in the same five-year period, i.e., between twenty and twenty-five. Consideration of the curves representing age incidence in the two groups reveals no significant difference. The group of "cystic adenoma" cases is too small to permit comparison.

No conclusion as to the influence of marital status upon the incidence of eccyclomastoma can be drawn. In the married group, women who have borne children, that is, whose breasts have undergone lactation hypertrophy, outnumber those without children, two to one.

Although no portion of the breast is exempt, eccyclomastoma has a predilection for the upper, outer quadrant. In the 236 cases with localization in one quadrant, 44.5 per cent of the lesions were in an upper outer quadrant.

A lump in the breast is the outstanding symptom, occurring in 83 per cent of cases. Pain occurred in 35 per cent and pain appearing or becoming intensified at the time of the menstrual period occurred in 15 per cent.

The average duration for all cases was thirty-six months; for the "fibro-adenoma" group, forty-four months; for the "intracanalicular myxoma" group, thirty-one months. There was no significant racial difference.

Movability of the mass was described in only 56 per cent of all cases, a figure which does not indicate the actual general occurrence of this characteristic.

In 15.75 per cent of the total number of cases the masses were multiple, involving one or both breasts. In 5.7 per cent of cases the mass occurred in a generally lumpy breast.

Dimpling of the skin was observed in 11 cases and retraction of the nipple in 9 cases. In those cases in which a preoperative diagnosis was made, the lesion was considered benign in 94 per cent.

The operation of choice was simple excision with a zone of surrounding breast. Where the size of the mass demanded or where the diagnosis of malignancy or possible malignancy was made, amputation or the complete operation was done (12 per cent).

One hundred and ninety-eight cases have been followed for more than one year after operation, and the results are tabulated.

The stroma of the breast consists essentially of two types, the interlobular which includes the interlobar, and the intralobular which includes the periductal and periacinar.

The importance of the changes occurring in the breast at puberty in connection with the production of eccyclomastoma and the possibility that many eccyclomastomata discovered late in life date from the time of puberty, have been emphasized.

It has been demonstrated that hypertrophy of breast tissue may be grossly unilateral or bilateral, may be diffuse or isolated and, if isolated, single or multiple in one or both breasts; and may consist of epithelial or connective tissue hyperplasia or any combination of the two. Also, it has been demonstrated that connective tissue hyperplasia may be interlobular or intralobular or both.

Encapsulation has been found to be a mechanical phenomenon, without significant bearing upon the pathology of eccyclomastoma. It is the author's belief that encapsulation is never entirely complete and that through their ducts the affected areas maintain connection with the remainder of the gland.

The general absence of uniformity of microscopic appearance throughout the affected areas has been emphasized.

The microscopic picture generally associated with the term "intracanalicular" may occur as a result of the involvement of an entire lobule as well. This type of pathology is, therefore, not necessarily intraductal.

Efforts to find a constant association between any particular type or consistency of connective tissue or any type of epithelial hyperplasia and rapidity of growth, duration of the mass, and age of the patient have been fruitless.

Epithelial cells lining ducts and acini with a tendency toward dilatation are likely to assume columnar form and acidophilic staining properties, and are not necessarily related to sweat glands or residual lactation.

In three cases fully differentiated squamous-cell epithelium and transitional stages between squamous and glandular types were found within areas of eccyclomastoma in which proximity to the skin could be definitely eliminated.

The cases of "fibro-adenoma," "intracanalicular myxoma," and "cystic adenoma" have been incorporated into one group in the absence of sufficient differentiating features, either pathological or clinical, to justify their separation.

It has been found that all the microscopic appearances commonly associated with chronic cystic mastitis, single and multiple cysts, papillary cystadenoma or Schimmelbusch's disease, dilatation of ducts and acini, desquamation, and the assumption of columnar form and acidophilic staining, are encountered within these benign areas of eccyclomastoma.

The evidence of response on the part of breast epithelium and connective tissue to hormonal stimuli, in the elaboration of which the ovary and anterior pituitary body at least must play a part, has been accepted. The fact that such hormonal control introduces a variable factor and a potential pathogenic agent has been stressed.

A second variable factor and potential pathogenic agent has been postulated, namely, a lack of uniformity of tissue behavior in response to stimulation. Evidence of such a lack of uniformity, even in processes considered normal, has been adduced.

It has been pointed out that every evolutionary or hyperplastic phase in any of the breast cycles is followed normally by an involuntionary phase, which in pregnancy and menstruation has its own proliferative aspects. It immediately follows that involution offers its own peculiar opportunities for abnormal behavior.

Any relationship between eccyclomastoma and carcinoma has been denied. There may be a slight tendency on the part of these masses to undergo sarcomatous change.

BLOOD SEDIMENTATION, The Significance of Repeated Red Cell Sedimentation Rate Determinations in Pulmonary Tuberculosis, Siltzbach, L. E. Am. Rev. Tubere. **29**: 673, 1934.

The E.S.R. curves (Cutler) were studied on 494 patients with pulmonary tuberculosis: 1170 individual tests were performed. The data so obtained were analyzed, and correlative studies with the constitutional and local manifestations of the disease were made. It was found that curves reflecting moderate and severe activity occur with greater frequency in patients with fever, rapid pulse, loss of weight, positive sputum, cavity-formation, extensive lesions, and extrapulmonary tuberculosis. The E.S.R. curve reflects the progress of the disease and a change in E.S.R. curve often precedes the change in clinical symptoms and signs. It is of value, therefore, in prognosis in pulmonary tuberculosis. In collapse therapy the E.S.R. may be utilized as a gauge of the effectiveness of the treatment. The use of E.S.R. curve determinations in routine sanatorium and private practice in tuberculosis is recommended.

MENINGITIS TUBERCULOUS, The Tryptophane Test in, Rosenblatt, M. B. Am. Rev. Tubere. **29**: 668, 1934.

The presence of a positive tryptophane test is strongly suggestive, but not pathognomonic, of tuberculous meningitis.

Hemorrhagic, purulent, and xanthochromic fluids give "false positive" reactions which are usually distinguishable from those obtained in tuberculous meningitis by the

purplish color. Indistinguishable positive reactions may be obtained with clear spinal fluids in which the protein is increased.

There is sufficient evidence to warrant the use of the tryptophane test as a routine procedure in the examination of the spinal fluid.

PREGNANCY, Studies in Pernicious Anemia of: Tropical Macrocytic Anemia as a Deficiency Disease With Special Reference to the Vitamin B Complex, Wells, L. Indian J. M. Res. **31**: 669, 1934.

Evidence is adduced and discussed that tropical macrocytic anemia is a simple condition of dietary deficiency.

The curative action of marmite (a yeast extract) in this condition already reported has been confirmed.

The nature of the hemopoietic factor was investigated by the use of various specially prepared fractions of the vitamin B complex.

By this means the recognized constituents of the vitamin B complex were excluded.

The hemopoietic fraction of marmite was found to be water-soluble and heat-stable in acid medium and not to be precipitated or inactivated by 80 per cent alcohol.

TUBERCULIN REACTION, Quantitative Study of, in Childhood Tuberculosis, Johnston, J. A., Howard, P. J., and Maroney, J. Am. Rev. Tubere. **29**: 652, 1934.

The reaction to tuberculin in the childhood type of tuberculosis has been studied throughout the evolution of the primary complex and found to follow a curve which rose to a high peak following the absorption of the parenchymal lesion and corresponding with the maximal involvement of the hilum lymph nodes, and then to decline with the diminution in size of these nodes, and their calcification. While this course of events is usually paralleled by peripheral blood changes associated with healing, the same tuberculin course was seen when x-rays showed absorption but the blood picture remained equivocal, or even showed retrogression. It is the authors' feeling that these data favor the concept that the allergic and immune phenomena, in so far as the latter are reflected in the peripheral blood, are relatively independent.

TRICHOMONAS VAGINALIS, Incidence and Treatment of, in Pregnancy, Glassman, O. J. A. M. A. **102**: 1748, 1934.

Vaginal discharge during pregnancy is often dismissed as a normal physiologic change but is frequently associated with Trichomonas vaginalis.

In a series of 309 pregnant patients personally examined, Trichomonas vaginalis was found in 20.7 per cent.

The symptoms are essentially the same as in the nonpregnant, and the diagnosis is easily made by a hanging drop.

The cases reported showed no increased puerperal morbidity.

The multiplicity of treatments indicates that a satisfactory method is not available, but a crystalline phenol powder has been used with good effect in this series.

Some cases are apparently cured spontaneously during labor and the puerperium.

The life cycle of the trichomonas requires further study, but in the meantime many patients may be given much relief from the annoying symptoms and leucorrheal discharge associated with this organism by more careful attention to the microscopic examination of the vaginal secretion.

B. DIPHTHERIAE, Differentiation of, Dockeray, G. C. Irish. J. M. Sc. **97**: 12, 1934.

The medium following is simple and easy to prepare and has been found of value in the isolation of B. diphtheriae.

Two Erlenmeyer flasks of about 500 c.c. capacity are taken and 150 c.c. of 5 per cent agar is put in one. This is sterilized in the autoclave, and while still liquid is used to prepare the medium. In the other flask is 150 c.c. of tryptic digest broth. To the broth

from 20 to 30 c.c. of sterile citrated human blood is added, followed by 120 mg. of potassium tellurite dissolved in a few cubic centimeters of sterile saline solution. The tellurite-blood-broth mixture is warmed to about 40° C. and is then poured into the melted agar, which has been allowed to cool to about 70° C. The mixture is heated in a water bath at 75° C. for ten minutes and, when cooled to from 50 to 55° C., is poured as plates.

INTRAVENOUS THERAPY, Study of Hyperpyrexia Reaction Following, Banks, H. M. Am. J. Clin. Path. 4: 260, 1934.

The reaction that follows intravenous administration of solutions is an entity characterized by an immediate and prompt rise of temperature in extremely high degree and usually associated with a severe rigor lasting about twenty to thirty minutes. This clinical picture is not to be associated with shock and is entirely separate and distinct from it. Intravenous reaction has a definite, specific, etiologic factor. This factor is the introduction of dead or living products of bacterial growth or cultures of, Pseudomonas scissa or ureae, into the blood stream of the individual.

These organisms were isolated in pure culture from the offending solution, reinjected into the individual and produced the same clinical picture. It was not possible to recover the organism from the blood stream of the individual suffering from a reaction, even though cultures were made at various times during the rigor.

The pyrogenic substance may be used as a therapeutic agent to produce hyperpyrexia.

TYROSINURIA, Origin and Significance of, in Disease of the Liver. Lichtmann, S. S. Arch. Int. Med. 53: 680, 1934.

Tyrosinuria was studied in health and in disease by the tyrosinase method. Its occurrence and estimation proved to be of significance in the diagnosis and prognosis of diseases of the liver and bile passages and in the interpretation of its mode of origin.

The quantitative observations in excretion of tyrosine confirm the view that the abnormal amounts of amino-acids excreted in the urine of patients with acute atrophy of the liver originate mainly from autolyzed liver tissue. While it is conceded that in acute diffuse degeneration of the liver the function of the liver in deamination may fail and contribute to the amino-aciduria, this factor plays only a minor rôle. Studies of the amino-nitrogen content of the blood and urine in some cases of subacute atrophy of the liver with tyrosinuria indicates that in this less acute form of degeneration of the liver tyrosine may appear in the urine when there is no apparent disturbance in the metabolism of amino-acids.

Observations on the rate of excretion of tyrosine are of diagnostic significance. Continuous massive tyrosinuria occurs only in cases of acute yellow atrophy with a rapid and fulminant course. Transitory minimal and moderate tyrosinuria occurs in cases of subacute atrophy of the liver, in degenerating neoplasm of the liver, in toxic degeneration of the liver and uncommonly in obstructive jaundice of long standing due to stone. Inflammatory lesions of the bile passages do not of themselves give rise to tyrosinuria.

Extrahepatic foci of autolysis, such as degenerating tumors of the lung or extensive sloughs of the skin, may give rise to minimal or moderate amounts of tyrosine in the urine.

The transitory nature of minimal or moderate tyrosinuria is demonstrated by repeated tests. During the phase of recovery from degeneration of the liver, the products of parenchymal autolysis have already been absorbed, and tyrosine vanishes from the urine, but with a fresh attack of degeneration of the liver, tyrosine reappears in the urine. In the stage of repair, namely in coarse nodular cirrhosis, or in the terminal stages of subacute yellow atrophy with a critical reduction in the functioning parenchyma of the liver, tyrosine may not be demonstrable in the urine.

Tyrosinuria may occur without jaundice either in cases of extrahepatic origin or in those of demonstrable disease of the liver.

Quantitative observations with respect to tyrosine are of prognostic value. In cases of minimal tyrosinuria the patients are more likely to recover, whereas cases of massive tyrosinuria terminate fatally in a short time. Mounting tyrosinuria indicates rapidly progressive degeneration of the liver. However, the absence of tyrosinuria in cases of degeneration of the liver does not warrant an optimistic prognosis.

CHOLESTEROL, Blood, and Thyroid Disease, Hurxthal, L. M. Arch. Int. Med. 53: 762, 1934.

Postoperative myxedema is accompanied by hypercholesteremia.

Subtotal thyroidectomy may be followed by hypercholesteremia without clinical myxedema. This is interpreted as a transient thyroid deficiency.

Subtotal thyroidectomy may be followed by low metabolic rates without hypercholesteremia. In these cases myxedema is seldom found.

Roentgen therapy can produce transient hypercholesteremia with or without clinical myxedema.

Removal of part of a thyroid gland affected by chronic thyroiditis may be followed by hypercholesteremia and myxedema.

Thyroid deficiency produces myxedema and hypercholesteremia, but at times myxedema may be clinically imperceptible.

Hypercholesteremia, when not explainable on any other basis, may be considered as possibly of thyroid origin, and is a rational indication for thyroid administration.

The finding of hypercholesteremia, in the absence of its few other common causes, points more specifically to thyroid deficiency than does the finding of a low metabolic rate. Finding both renders the possibility of thyroid deficiency extremely likely.

The relationship between the blood cholesterol and the basal metabolism is usually reciprocal when they undergo change as the result of variations in the activity of the thyroid gland or thyroid substance in the body.

The blood cholesterol provides another variable which may be used as a guide in the treatment of thyroid disease.

IRON, Constancy of, in the Blood Plasma and Urine in Health and Disease, Marlow, A., and Taylor, F. H. L. Arch. Int. Med. 53: 551, 1934.

The iron content of the blood plasma of normal persons varies between 0.4 and 0.7 mg. per hundred c.c., while that of the urine ranges from 0.03 to 0.8 mg. per twenty-four hours.

The iron content of the blood plasma and urine of three patients with hypochromic anemia due mainly to chronic loss of blood and of two patients with pernicious anemia soon after remission had been inaugurated was found to vary within normal limits. The oral administration of ferric ammonium citrate to these patients and to normal persons caused no definite increase in the iron content of the blood plasma or of the urine. However, in the normal person studied there was perhaps a slight increase in the urinary excretion of iron following the oral administration of this substance in an acid-buffered medium.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor,
Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

A Synopsis of Medicine*

WHILE it is not the purpose of a "synopsis" to serve as a textbook of medicine, as a source of ready reference and a means of review, such works have an eminently practical and valuable place in the practitioner's library.

Tidy's is among the best known of such works so that it is not surprising to see a new sixth edition which has been extensively revised to keep abreast of the new and important advances which have been made since the last edition in 1930.

The revision has been thorough and extensive, so much so that the book has been entirely reset, the new material making an addition of about twenty-five pages.

Among the sections in which many changes have been made are those on Deficiency Diseases, Diseases of the Parathyroid Glands, and Diseases of the Bones.

Nineteen new articles have been added and extensive changes made throughout the text in general.

The book can be recommended confidently to the practitioner as reflecting the status praesens of the entire field of medical practice.

Tumors of the Female Pelvic Organs†

IN THIS book Dr. Meigs presents a survey of the present knowledge of the various tumors, both benign and malignant, that occur in the female pelvic organs.

While the material is based chiefly upon a series of cases from the Massachusetts General Hospital, these have been so carefully studied and verified and the pertinent literature so thoroughly reviewed, that the book may justly be regarded as an extremely valuable and authoritative presentation of the subject.

In view of the extensive and almost bewildering accumulation of literature in this field, this book should be heartily welcomed by all concerned with it, as who should not be.

The plan upon which the volume is constructed is eminently practical. Beginning in each instance with a survey of the present pathologic knowledge of the tumors in each organ, Dr. Meigs then discusses in correlation with these facts the clinical signs and symptoms and finally the appropriate methods of treatment and their results.

The style is clear and simple and the volume bears the stamp, not only of a thorough acquaintance with the literature, marked by discriminating analysis, but also of an extensive and well-evaluated practical experience.

Both the author and the publishers are to be congratulated on the excellent illustrations.

To the pathologist, the surgeon, the roentgenologist and the practitioner Dr. Meigs's book should come as a standard reference text not likely to be displaced for some time to come.

The book may be highly commended as a contribution of great practical value and importance.

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*A Synopsis of Medicine. By Henry Letheby Tidy, Physician to St. Thomas' Hospital, London, etc. Ed. 6, cloth, pp. 1112. Wm. Wood & Co., Baltimore.

†Tumors of the Female Pelvic Organs. By Joe Vincent Meigs, M.D., Instructor in Surgery, Harvard Medical School, with a foreword by Robert B. Greenaugh, M.D., President-elect, American College of Surgeons, 1933-34. Cloth, pp. 533, 261 illustrations. The Macmillan Company, New York.

Physical and Clinical Diagnosis*

THAT this book has achieved twenty-four German and two English editions will not be surprising to the reader for in small compass it covers, and covers clearly and well, the entire field of physical and clinical diagnosis.

Intended to serve as a succinct and compact compendium for use as a handbook of ready reference, it achieves its purpose excellently well. While succinct it is not laconic and comprises a wealth of material of practical value. That so much can be contained in so little space is largely due to the format, the volume being pocket size. Unlike so many "pocket manuals" the type is of comfortably readable size and character.

This book may be highly recommended.

Die operative Technik des Tierexperimentes†

THE second edition of this manual which comprises the fifth division, Part 3 c of Abderhalden's "Handbuch der biologischen Arbeitsmethoden" will be found of inestimable value for those who are engaged in the laboratory sciences. The author describes the habits and care of the ordinary laboratory animals, the various anesthetics and their mode of administration, and the question of asepsis quite thoroughly. Detailed descriptions are given of the various operative procedures on almost every organ of the body. The text is replete with excellent illustrations.

H. B. HAAG.

*Physical and Clinical Diagnosis. By Dr. Otto Seifert, late Professor of Medicine, Wuerzburg, and Dr. Friederich Muller, Professor of Medicine, II Clinic, Munich, Second English Edition, Authorized translation by E. Cowles Andrus, M.D., Associate Professor of Medicine, Johns Hopkins Hospital. Cloth, pp. 561, 152 figures, 4 color plates. J. B. Lippincott & Co., Philadelphia.

†Die operative Technik des Tierexperimentes. (The Operative Technic of Animal Experiments.) By H. F. O. Haberland, Dr. med., Professor of Surgery, University of Köln. 434 pages, with 382 illustrations. Urban & Schwarzenberg, Berlin, 1934.

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EDITORIAL

The Mills of the Gods and Anesthesia

THE mills of the gods grind slowly. And there are perhaps few fields of human endeavor in which this has been better illustrated than in that of anesthesia, for the long and despairing silence of more than two thousand years between the discovery of opium and the first practical use of ether, nitrous oxide and chloroform to relieve human suffering serves to illustrate in a singularly striking manner the weakness and the strength, the failures and the successes of the healing art. It is interesting to note that even so late as 1839 the great French surgeon Velpeau was able to write that "to escape pain during surgical operations is a chimera for which no solution need be expected within our time." This was only three years before Long used ether in a surgical operation. And it certainly behooves the medical profession to anxiously view the future in hopefulness that history may thus tend to repeat

itself with reference to some of the other great stumblingblocks that have baffled medical science for much more than two thousand years. But notwithstanding the agonies and horrors through which the human race passed before anesthesia got its start, it now seems certain that no fault can be found with the speed of advancement nor with the volume of knowledge by which anesthesia has been enriched in very recent years. Indeed it seems very probable that the specialty of anesthesia is now further advanced and has attained a more certain and satisfactory command of its particular problems than is the case with any other specialty in the whole field of medicine.

It has now been just twenty years since a paper on the invention of the closed system for anesthesia with absorption of carbon dioxide constituted the first article[1] which introduced this JOURNAL to the medical profession in October, 1915. In the meantime ethylene and cyclopropane have been added to the list of gaseous anesthetics. And vinesthene (divinyl ether) which has much in common with the gases, has been introduced. It now seems certain that hereafter a constantly increasing number of anesthesias produced by these anesthetic agents will be carried out by the use of the carbon dioxide absorption method. The increased safety (for these anesthetics are explosive and inflammable), the reduced cost and the greatly improved type of anesthesia made possible by this method will insure its eventual almost universal adoption.

Trichlorethylene,[2] which has general anesthetic properties and is neither explosive nor inflammable, may be so cheap that it can better be used without the closed system, but even here the relative advantages are yet to be investigated.

While none of these anesthetics are absolutely ideal still they represent a great stride forward. The introduction of the newer barbiturates and similar hypnotics as basal anesthetics, or in some instances, as the sole anesthetic agent used, also represents a distinct advancement in this field.

Cyclopropane, the newest gas to be used extensively as a general anesthetic, is a three carbon cyclic compound isomeric with propylene and having the formula C_3H_6. It was first prepared by Freund[3] in 1882, while Lucas and Henderson[4] in 1929 carried out a series of animal and chemical experiments and announced the general anesthetic properties of the gas on animals and also the observation that only 9 per cent of cyclopropane in oxygen would produce unconsciousness when inhaled by these workers. Ten to 20 per cent of the gas in oxygen produced satisfactory anesthesia, with but little change in the vital physiologic functions of the body. Waters and Schmidt as early as 1932 were working with the gas, and in 1934 Stiles, Neff, Rovenstine and Waters[5] published a most interesting report on four hundred and forty-seven anesthesias with cyclopropane. With the especial caution that the use of the gas should be regarded as still in the experimental stage for another year these workers reported satisfactory results in this series of anesthesias. The gas acts quickly and powerfully and complete relaxation can nearly always be obtained. Waters and his coworkers failed in only two of the four hun-

dred and forty-seven cases mentioned above. In one of these ether produced relaxation only after a huge amount had been administered. The gas is more rapidly flexible than ether and consequently the depth of anesthesia can be regulated much more quickly. Consciousness is usually regained in four or five minutes though the occasional patient may require as long as ten to twelve minutes. This recovery time corresponds well with that following trichlorethylene. Cyclopropane is apparently rapidly exhaled from the body and when the patient revives he thereafter remains awake. Postoperative complications compare favorably with those of other anesthetics in general use. It seems probable that in this respect trichlorethylene may be freer from postoperative nausea, etc., than cyclopropane or most other anesthetics.

Cyclopropane is explosive but less so than ethylene. There is also a difference in the relative explosiveness of various percentage mixtures of each of these gases with oxygen. A good anesthetic mixture of ethylene and oxygen will hardly explode although it may burn like illuminating gas. But a 10 or 15 per cent mixture of ethylene with 85 or 90 per cent of oxygen is very explosive. On the other hand it appears that the most explosive mixture of cyclopropane with oxygen is in the neighborhood of 50 per cent of each gas. The best anesthetic mixture of cyclopropane with oxygen, however, is within the limits of 10 to 20 per cent of the gas with oxygen. This mixture will hardly explode, but 25 per cent cyclopropane with 75 per cent oxygen is moderately explosive. From this it will be seen that the anesthesia is started with a mixture that is not explosive, and it is only as more and more of the gas is given to increase the depth of the anesthesia that a really explosive mixture is produced. Just the reverse of this happens in starting off an anesthesia with ethylene. Here one may hurry to increase the percentage of ethylene to get past the most dangerous mixture, while with cyclopropane one may hold back the gas to keep within safer limits.

Cyclopropane appears to be too powerful and too rapid in its action to render its use safe within the medical profession at large until a period of education in its action and administration can be carried out. This is especially true for those who have not had special training in the field of anesthesia. For the gas produces a rapidly moving series of symptoms which differ considerably in several respects from those produced by the other older anesthetic agents. Trichlorethylene[6] on the other hand acts more slowly, there is often a rather marked excitement stage and the patient does not seem to be so profoundly depressed. But the *(central)* analgesic properties of the drug, even in very small doses, are often well marked as may be seen in its use to relieve the pains of tic douloureux,[7] in about 10 or 11 per cent of which patients may be permanently cured under its use.

The splendid investigations which are now being vigorously carried forward along these lines by such workers as Woodbridge, Waters, Hammond, Leake, Bourne, Herb, Lundy, Luckhardt, Rovenstine, Guedel, Brown, and many others can leave no doubt but that at last the mills of the gods are grinding rapidly as well as "exceeding fine" so far as anesthesia is concerned.

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—*D. E. J.*